

Understanding the physiological role of the cerebellum and motor cortex
on human motor learning

by
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Abstract

Many of our daily life activities, such using a new computer or playing sports rely on the acquisition and retention of specific movement patterns. Our ability to learn these patterns depends on multiple behavioral and neuronal processes. While animal research has described learning-related plastic changes within the cerebellum and primary motor cortex (M1), little is known how these changes relate to learning different types of human behavior. In this dissertation, we used non-invasive brain stimulation to assess neural excitability in these brain regions following motor adaptation and skill learning. In chapter 2, we investigated whether excitability changes occurring in the cerebellum are somatotopy-specific in the presence or absence of adaptive motor learning. We used transcranial magnetic stimulation (TMS) to assess cerebellar excitability and found that learning elicited changes for not only a trained effector, but also for an uninvolved effector, likely related to inter-effector transfer of learning. However, when assessing excitability during movement preparation, where no learning occurs, we found modulation was effector-specific, indicating that learning-related changes in cerebellar excitability follow a somatotopy specific rule. Subsequently we studied whether this cerebellar physiological mechanism also extends learning a skill, a behavior known to induce long-term plasticity (LTP) changes in M1. In chapter 3, we used both TMS and transcranial direct current stimulation to explore cerebellar and M1 mechanisms during different stages of motor skill learning. We found a reduction in cerebellar excitability early in skill learning, but not late. On the other hand, changes in M1 long-term potentiation (LTP)-like plasticity only occurred after a significant amount of training had taken place. While this hints towards an important temporal interaction in the physiological role of the cerebellum and M1 when learning a novel skill, it remained unclear if this result was related to acquiring distinct motor components that constitute the skill. The motor skill participants learned involved integrating how to interact with a new device and environment (sensorimotor map), along with a sequence

movements. In chapter 4, we deconstructed the skill task to identify distinct physiological contributions of the cerebellum and M1 associated to learning each skill component. We found that learning the sensorimotor map, reflecting the dynamics of the skill, only elicited changes in cerebellar excitability, whereas learning the sequence of movements resulted in both cerebellar excitability and M1 LTP-like plasticity changes. These results indicate that learning the different components that constitute a motor skill engages the cerebellum and motor cortex in a concerted manner.

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Chapter 1 – Background and Introduction

1.1 General Introduction

Humans have the extraordinary ability to acquire and perform complex motor behaviors that characterize our way of living. Take for example, Argentine superstar Lionel Messi's ability to create space on the football pitch by dribbling his way past several defenders, followed by striking the ball past the goal keeper for a spectacular goal. We are shocked by how he is able to seamlessly control and perform this impressive skill with the ball, which required several years of training and experience to perfect. However, we take for granted how even the most routine and effortless movements we make, such as showering and getting dressed in the morning, also requires our brain to coordinate hundreds of muscles to prevent ourselves from falling and potentially getting injured. This is remarkable given the amount of computations our brain undertakes to make these movements accurately and with little conscious effort. The complexity of performing these movements becomes evident following illness or neurological damage that results in motor impairments.

As in the case of stroke survivors, this results in difficulty to perform many daily life activities, and ultimately reduces the quality of life and constant need for assistance and rehabilitation. While the value of rehabilitation aimed to account for these impairments is well recognized, recovery of normal motor function is often limited with current therapeutic strategies. Thus, it is critical to gain a better understanding of the neurophysiological bases and neural substrates underlying how we learn new behaviors, in order to provide insight for improving future rehabilitation interventions. Specifically, we need to understand how healthy individuals are capable of learning and retaining new motor behaviors to not only broaden our knowledge of how

the central nervous system works, but to also translate this information to a clinical setting to perhaps augment motor relearning and recovery.

1.2 Overview of Sensorimotor Learning

In the field of system motor neuroscience, sensorimotor learning can be categorized into two types of behavior that involves: (1) a recalibration of a previously learned movement, such as adjusting your hand to use a friend's computer mouse for the first time; (2) acquiring an entirely new motor control policy, such as learning to play an instrument.

In more detail, motor adaptation refers to how our central nervous system is able to modify well-known movements by making errors and correcting them on subsequent movements (Bastian 2008). The overall goal in this type of learning is to regain former levels of performance in new settings. This sensorimotor recalibration process is critical for performing movements in our daily life, since we often encounter different environments that require flexibility of our movement patterns. For example, imagine the scenario when you rent a car for vacation travel. It is likely the brake pedal sensitivity of the rental car responds differently to your own car does, resulting in either braking too suddenly or getting dangerously close to the car in front of you. Our brain is capable of adjusting (i.e. recalibrating) our foot movements to account for the new brake pedal sensitivity within minutes of being on the road; however, when you first return back to using your own car, you initially misestimate how hard you need to press on the breaks due to the prior experience of driving with the rental car.

The other type of learning studied here refers to motor skill learning, which involves acquiring a series of de novo movement patterns that are refined through continual practice and reinforced by successful goal completion. We engage in skill learning when we first interact with a new object or environment, such as playing a new instrument or taking on a new sport. For instance, imagine trying to execute a tennis serve for the first time. You not only have to learn a new sequence of

movements (i.e. tossing the tennis ball followed by a fluid overhead swing), but you also have to gain an understanding of how to strike the ball with your racket. Both of these components are critical to master in order to successfully serve the ball in the opposite player's court. Unlike motor adaptation, learning this type of behavior occurs on a timescale of anywhere between minutes to years, and is typically operationalized in laboratory settings as shifts in the trade-off between speed and accuracy for a task when no systematic perturbation is present (Reis et al., 2009). Additionally, motor skill acquisition is also characterized by a reduction in movement variability with practice, whereas the faster motor adaptation process is categorized by reductions in errors (Shmuelof et al., 2011). For instance, a professional tennis player is capable of consistently serving the ball on a specific location of the opponent's court and will show less temporal-spatial fluctuations in his serves when compared to a novice player.

1.3 Overview of different forms of motor learning and their neural substrates

Several lines of work have recently demonstrated that sensorimotor learning involves at least four distinct forms of learning that each are capable of contributing to motor adaptation and skill (Krakauer and Mazzoni, 2011; Haith and Krakauer 2013; Taylor and Ivry 2013). This includes, but is not limited to, error-based, use-dependent plasticity, reinforcement and cognitive strategies, each thought to involve different neuronal substrates.

Error-based learning refers to the process of developing forward internal models (i.e. a model capable of predicting the consequences of a motor command) by reducing errors that relay the difference between predicted sensory consequences of a motor command and the resulting sensory feedback. The presence of this error, known as sensory prediction error, is important because the motor system uses this discrepancy to immediately adjust the subsequent motor output. Evidence from several lines of work have implicated the cerebellum as a critical structure involved in error-based learning (Miall and Wolpert, 1996; Diedrichsen et al., 2005; Tseng et al.,

2007; Criscimanga-Hemminger et al., 2010). For instance, research on patients with cerebellar damage have shown that adjusting internal models to reduce sensory prediction errors heavily depends on the cerebellum (Tseng et al., 2007; Taylor et al., 2010; Izawa et al., 2012), while both animal and human physiological studies have indicated that this type of learning leads to plastic change within the cerebellum (Medina and Lisberger, 2008; Yang and Lisberger, 2014a&b; Jayaram et al., 2011; Schlerf et al., 2012).

On the other hand, reinforcement learning refers to selecting an action based on success or failure feedback. In other words, a particular movement that leads to a successful outcome is reinforced, whereas an unsuccessful outcome is avoided. This form of learning relies on the exploration of different motor commands in order to maximize successful outcomes. Similar to error-based learning, reinforcement learning is driven by comparing an expected and observed outcome. However, learning in this mechanism is driven by a reward prediction error that relates to the probability of an action resulting in a success (Sutton and Barto, 1998), thought to rely on circuits involving the basal ganglia (Schultz, 2006; Ramayya et al., 2014). In addition, work has also suggested a link between reinforcement learning and primary motor cortex (M1). For instance, animal work has demonstrated that dopaminergic neurons in ventral tegmental area (a major midbrain nucleus projecting to M1) contributes to M1 plasticity (Luft and Schwarz, 2009; Hosp et al., 2011) and play a role in motor skill learning (Hosp et al., 2011; Kawai et al., 2015; Rioult-Pedotti et al., 2015).

Beyond learning from sensory and reward prediction errors, our motor behaviors are also influenced by the history of our motor actions. In other words, movement repetitions or performing training, memory traces of that action can be identified. Repeating movements to a particular target has been shown to bias subsequent movements towards that direction (Huang et al., 2011; Verstynen and Sabes, 2011), or increase the likelihood of a movement (Diedrichsen et al., 2010). This repetition-induced bias in movements, known as use-dependent (plasticity)

learning, can occur independently of training goals, but are dependent on the history of prior executed movements. This form of learning is thought to originate in the primary motor cortex (M1), as repetitions of movement leads to plastic reorganizational changes in M1 (Classen et al., 1998; Butefisch et al., 2000; Dayan and Cohen, 2011). For instance, when individuals repeatedly perform thumb movements in a particular direction, transcranial magnetic stimulation of M1 following training is more likely to elicit responses in the trained direction (Classen et al., 1998). Use-dependent learning has previously been interpreted as a result of Hebbian-like changes in M1 (De Xivry et al., 2011) and interestingly has been shown to be augmented by reinforcement mechanisms (Mawase et al., 2017).

Furthermore, it is critical to point out that these different forms of motor learning, and their associated brain regions, contribute to how we learn new behaviors. For example, cognitive, reinforcement and use-dependent plasticity mechanisms are capable of contributing to motor adaptation, a behavior traditionally thought to rely on error based learning (Diedrichsen et al., 2010; Huang et al., 2011; Izawa and Shadmehr, 2011; Haith and Krakauer, 2013; Taylor and Ivry, 2011, 2014). However, we argue that the relative weights of how these forms of learning contribute to the learning process change throughout the course of learning a specific behavior. For instance, it was shown that disrupting M1 with transcranial magnetic stimulation only impairs motor adaptation when performance plateaus (Orban de Xivry et al., 2011), indicating that repetition of the same motor command plays a significant role in how much M1 contributes to motor adaptation. Consistent with this interpretation and the hypothesized role of the cerebellum, applying anodal cerebellar transcranial direct current stimulation during motor adaptation speed up the error correction rate, whereas the same stimulation applied over M1 enhanced motor retention of the recently behavior (Galea et al., 2011). Interestingly, similar stimulation effects have been found in motor skill learning when targeting these specific brain regions (Reis et al., 2009, Cantarero et al., 2015).

1.4 Mechanisms of Learning in the Cerebellum

The ability of the cerebellum to map sensory and motor signals from descending cortical pathways and ascending peripheral pathways onto identified cell types has led to several lines of work investigating the roles of cellular and synaptic plasticity mechanisms in motor learning (for review, see D'Angelo et al., 2016). While it is known that the cerebellum is a highly plastic region critical for motor learning, the underlying cellular and synaptic mechanisms are still misunderstood. However, the theory proposed by Marr (1969) and Albus (1971) suggests that the cerebellar cortex is suited for error-based learning. This theory suggests that signal carrying information of planned or ongoing movement is relayed to the mossy-fiber-parallel-fiber system, whereas the climbing fiber relays a teaching a performance error signal to the cerebellar cortex. When parallel fibers and climbing fibers activation coincide, long-term depression (LTD) or decrease in synaptic strength at parallel fiber-Purkinje cell synapse occurs. Through this process, the cerebellar output associated with the same movement (parallel fiber signal) is adjusted. Support for this hypothesis includes evidence from animal studies showing complex spike discharge associated with learning smooth pursuit adaptation (Medina and Lisberger, 2008) as well as when adapting reach movements to unexpected loads (Gilbert and Thach, 1977). Interestingly, one study found that prolonged bursts of climbing fiber activity, scaling with movement error, induce additional complex spiking in Purkinje cells (Yang and Lisberger, 2014). Furthermore, they showed the size of the error proportionally increases calcium levels and the expression of LTD. However, there are several lines of work demonstrating enhanced simple and complex spike discharge (e.g., potentiation of Purkinje cells) can also occur during learning (Bertheir and Moore, 1988; Ojakangas and Ebner, 1994).

Evidence from non-invasive brain stimulation work in humans has also indicated neurophysiological changes in this region following error-based learning. For instance, changes in cerebellar excitability following human locomotor adaptation were found proportional to the

amount individuals learned (Jayaram et al., 2013). Moreover, when learning a visuomotor adaptation, cerebellar excitability changed only when rotations were introduced abruptly (i.e. large sensory prediction error), and returned back towards baseline values when movements were successfully calibrated (Schlerf et al., 2012). Together, these results suggest a link between human cerebellar excitability changes and error-based learning.

1.5 Mechanisms of Learning in the Motor Cortex

The primary motor cortex (M1) is an essential structure for learning and retention of motor learning. Both the anatomical and physiological organization of M1 provides the capability for motor representations. It is well known that M1 is organized as motor maps consisting of somatotopically arranged representations of muscle patterns that allow for the control of several joint and muscle groups (for review, see Hosp et al., 2011). Since the intra-cortical connections within M1 are assembled through extensive horizontal pathways spanning layers II, III, and V, it has been suggested that this organization can account for the coordination of complex multi-jointed movement sequences (Kishore and Popa, 2014). This is critical for learning new motor skills, since developing them requires the selection and repetition of specific muscle movement patterns, thought to involve strengthening pathways of select intra-cortical connections while weakening others. This process includes, but may not be limited to, changes in the strength of synaptic connections, synaptic vesicular pool, and in the formation and eliminations of synapses, dendrites and axons.

Indeed, acquisition and retention of motor skill can lead to both functional (Karni et al., 1994; Plautz et al., 2000; Rioult-Pedotti et al., 2007) and structural (Kleim et al., 2002, 2004; Kolb et al., 2008) changes occur in M1. The physiological mechanisms that contribute to these changes are dependent on the times scale of learning. For example, reorganization of movement representations in M1 of rodent (Kleim et al., 1998a; Remole et al., 2001) and squirrel monkeys

(Nudo et al., 1996) is thought as slower process that likely depends on the restructuring of M1 microcircuitry. Previous studies have shown that motor skill learning, induces structural changes of M1 dendritic spines and synaptic morphology (Jones et al., 1999; Kleim et al., 2002b; Harms et al., 2008; Xu et al., 2009; Fu et al., 2012), enhances synaptic efficacy in M1 (Rioullet-Pedotti et al., 1998; Hodgson et al., 2005), and occludes further LTP while enhancing LTD in these connections (Rioullet-Pedotti et al., 1998, 2000, 2007). Moreover, dopamine may also play an important role in skill acquisition, as in-vivo work in rats recently showed that blocking mesocortical dopaminergic signaling impairs motor skill learning and reduced LTP within M1 (Molina-Luna et al., 2011).

Evidence for neurophysiological changes following motor learning in human studies using non-invasive brain stimulation have shown parallel results to animal work. Repeating the same movement pattern, in the context of learning, increases corticomotor excitability and promotes reorganization of movement representations (Pascual-Leone et al. 1995; Classen et al. 1998; Muellbacher et al. 2001; Perez et al. 2004). Motor learning also modulates LTP-like plasticity (Stefan et al., 2004; Ziemann et al., 2006) and occludes further LTP-like induction (Rosenkranz et al., 2007; Cantarero et al. 2013a&b; Avazino et al., 2015), an effect found related to motor retention (Cantarero et al., 2013a&b). Interestingly, administering dopamine D2 receptors-blocking drugs in humans is capable of blocking LTP-like plasticity (Luft and Schwarz, 2009), and M1 plasticity induced by TMS protocols is severely altered in Parkinson's patients off dopaminergic medication (Morgante et al., 2006; Ueki et al., 2006; Kishore et al., 2012).

1.6 Assessing Neurophysiological biomarkers in humans

1.6.1 General introduction to transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation tool capable of stimulating distinct regions of the human brain by using induced currents. During a TMS

procedure, an electromagnetic stimulating coil, consisting of wire loops encased in plastic, is placed on top of the scalp. For the magnetic stimulation, a short current pulse (~100 us) applied to the coil of wire induces a magnetic field that passes perpendicular to the current flow in the coil. The magnetic field (~ 1.5-2 tesla) penetrates through the scalp and induces an electric field perpendicular to the magnetic field. TMS is thought to stimulate at sharp bends of the axon where thresholds to incoming current are lower. The effects of TMS depend on parameters including stimulus amplitude, pulse waveform, pulse duration, and the shape and orientation of the coil. For instance, holding the coil at an angle of 45 degrees to the medial-sagittal plane over the motor cortex, preferentially activates pyramidal cells transynaptically to induce indirect waves (I-wave) over direct activation (D-wave) of descending axons. What makes TMS an attractive method for scientific research is that it can be used to either assess or modulate cortical excitability, inhibition, connectivity between distinct brain regions, and plasticity.

Applying a TMS pulse over M1 is capable of producing responses in contralateral muscle activity, known as a motor evoked potentials (MEPs). Of relevance to the studies presented here, MEP amplitudes are recorded with surface electromyography to quantify the level of cortico-spinal excitability. Thus, changes in the state of cortico-spinal excitability can be assessed with TMS. The amplitude of MEPs resembles a complex signal that is compounded at the spinal cord, consisting of a series of descending cortico-spinal volleys (for review, see Hallet 2007; Di Lazzaro and Rothwell, 2014). The different components include the D-wave, which reflects direct activation of pyramidal axons part of the cortico-spinal tract, followed by several I-waves (1 ms) that reflect indirect depolarization of the same axons via tran-synaptic connections (Ziemann and Rothwell, 2000). Changing the coil orientation (with stimulation intensity being held constant) can affect type of volley elicited and the size of the MEP amplitude, indicating stimulation of different neural subpopulations. Moving the coil over the surface of the scalp within the region of M1 is capable of stimulating different muscles of limbs, such as the hand and leg. Targeting a

specific muscle requires placing the TMS coil over the “hot spot” or area of the scalp over M1 that when stimulated, elicits a response of the targeted muscle.

1.6.2 Using TMS to assess the connectivity between the cerebellum and M1

Ugawa and colleagues (1995) were the first to demonstrate that either a high-intensity electrical pulse or magnetic stimulation over the inion of the cerebellum decreases cortical-spinal excitability of M1. Specifically, they showed that delivering a conditioning TMS pulse over the cerebellum 5 to 7 ms prior to delivering another TMS pulse over M1, reduced MEP amplitudes relative to trials with no cerebellar stimulation (Ugawa et al., 1995; Pinto and Chen, 2001, Daskalakis et al., 2004). This effect of cerebellar stimulation, known as cerebellar inhibition (CBI), has been suggested to result from activation of Purkinje cells inhibiting the dentate nucleus, which in turn has excitatory projections through ventrolateral thalamus to M1 (Celnik 2015). This interpretation is supported by work done in patients with lesions in the cerebellar-thalamic-cortical pathway or patients with atrophy of cerebellar hemisphere showing no CBI (Ugawa et al., 1997; Shirota et al., 2010; Kikuchi et al., 2012). It is also consistent with results demonstrating deep brain stimulation of the ventrolateral thalamus in essential tremor patients could influence CBI responses (Molnar et al., 2004). The double-cone TMS coil, designed to stimulate deeper tissue, can effectively stimulate the cerebellum and is the most effect at eliciting CBI (Hardwick et al., 2014). However, one concern with cerebellar stimulation includes inducing other non-cerebellar effects in addition to activation of this pathway. Werhahn and colleges were able to demonstrate the appropriate stimulation parameters to assure that the effects of cerebellar stimulation are not directly due to activation of the brainstem or anti-dromic activation of the cortico-spinal tract. Together, these investigations demonstrate that the presence of CBI is representative of cerebellar excitability, at least assuming M1 excitability does not change.

Beyond paired-pulse stimulation intensity and timing, the response of CBI is also influenced by other critical factors. For instance, CBI is reduced during the voluntary contraction of the target muscle (Pinto and Chen, 2001), but not when contracting surround muscles (Panyakaew et al., 2016). On the other hand, CBI decreases for both active and surround muscles when recorded at movement onset (Kassavetis et al., 2011). Therefore, it is important to consider the effects of muscle state if measuring CBI prior to or during movement initiation. Moreover, our group has also shown that the magnitude of CBI is also affected by handedness, where a stronger response is found for the dominant side relative to the non-dominant side (Schlerf et al., 2014). Here, we found an interesting relationship between CBI and reach movement variability, where stronger connectivity was correlated with better arm –reach precision. Stimulation of the cerebellar hemisphere is also capable of modulating both excitatory and inhibitory intra-cortical circuits in M1. Specifically, SICI was found to reduce while ICF increased in the presence of CBI (Daskalakis et al., 2004). In the context of motor learning, CBI has been found to change during error-dependent motor tasks (Jayaram et al., 2011; Schlerf et al., 2012) and when observing or practicing a sequential task (Torrerío et al., 2011). Interestingly, Jayaram and colleagues found that participants experiencing a larger magnitude of adaptation (i.e., greater degree of learning) expressed greater reduction in CBI. Together these studies suggest a link between error-based motor learning and changes in human cerebellar excitability. In this dissertation, we considered changes in cerebellar excitability, probed by cerebellar-M1 stimulation, as our physiological marker representing cerebellar contributions to learning.

1.6.3 General introduction to transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) is another form of non-invasive brain stimulation technique where a weak current (~1-2 mA) delivered through the skull via two small electrodes, can modulate cortical excitability. Unlike the application of TMS, tDCS does not directly induce neuronal firing of action potentials. Rather, animal studies have shown that polarizing current can

alter the resting membrane potential of neurons, and induce excitability changes in spontaneous neuronal discharges and evoked potential amplitudes for up to five hours (Creutzfeldt et al., 1962; Bindman et al., 1964; Purpura and McMurtry, 1965). In humans, current induced excitability changes in M1 can be detected for 90 minutes post-stimulation (Nitsche and Paulus, 2001), however the duration of these effects are dependent on stimulation intensity and duration (Bindman et al., 1964; Nitsche and Paulus 2000, 2001; Liebetanz et al., 2006).

1.6.4 Using tDCS to assess M1 long-term plasticity-like mechanisms

The lasting effects on cortical excitability changes following tDCS application are thought to involve synaptic plasticity mechanisms similar to long-term potentiation (LTP). Indeed, a recent study demonstrated that in vivo tDCS induced a robust enhancement in synaptic plasticity, including LTP changes in the hippocampus. In humans, antagonizing N-methyl d-aspartate (NMDA) receptors prevents the induction of long-lasting after effects (Nitsche et al., 2003a), while agonists increase the duration of the after-effects (Nitsche et al., 2004). Animal studies have confirmed the dependency of NDMA receptors (Fristch et al., 2010; Rohan et al., 2015), and extended this knowledge by showing that direct current stimulation LTP was absent in BDNF and TrkB mutant mice (Fristch et al., 2010). Interestingly, Val66Met, a common nucleotide polymorphism known to affect BDNF secretion in humans, decreases the response to tDCS (Puri et al., 2015) and affects performance of memory and learning tasks (McHughen et al., 2010; Dincheva et al., 2012; Taschereau-Dumouchel et al., 2016). Furthermore, the involvement of glial cells, and astrocytic Ca²⁺ has been recently related to anodal tDCS aftereffects through astrocytic Ca²⁺ / IP3 signaling (Monai et al., 2016), a pathway critical to synaptic plasticity (Taufiq et al., 2005). Together, these findings provide a strong rational for using tDCS as an LTP-like inducing protocol, with the potential to either assess the LTP-like plasticity changes or modulate motor behavior.

Numerous studies have demonstrated that combining motor practice with anodal tDCS (AtDCS) over M1 augments learning (for review, see Ammann et al., 2016). The effects of tDCS on motor learning are thought to occur through a mechanism similar to LTP (Reis and Fristch, 2011), given that overlapping LTP-like processes have been suggested to be involved in both AtDCS and motor learning (Fritsch et al., 2010; Cantarero et al., 2013). For example, a recent study demonstrated that AtDCS over M1 also modulates the effects of use-dependent plasticity (Rroji et al., 2015), where learning is believed to occur through strengthening synapses via LTP-like mechanism (Classen et al., 1998; Butefisch et al., 2000; Rosenkranz et al., 2007). Moreover, Cantarero and others (2013) showed that potentiating effects of AtDCS on M1 excitability are occluded following motor skill learning when compared to AtDCS-induced effects measured in the absence of training. Interestingly, the authors also provided evidence of a link between occlusion of LTP-like plasticity and skill retention. In this dissertation, we used the same the protocol as Cantarero and others (2013a&b) that combines AtDCS with TMS to assess the presence of occlusion of M1 LTP-like plasticity following motor learning. As such, we used occlusion of M1 plasticity to represent as a physiological marker of M1 contributions to learning.

1.6.5 Applying tDCS to the cerebellum and its effects on motor learning

Given the importance of the cerebellum in both cognitive and motor domains, the amount of studies applying tDCS over the cerebellum has increased dramatically (Grimadli et al., 2016). Numerous studies have shown that applying cerebellar tDCS can enhance a variety of learning tasks including: motor adaptation (Galea et al., 2011; Jayaram et al., 2012; Block and Celnik 2013; Herzfeld et al., 2014; Hardwick and Celnik 2014; Panico et al., 2016), skill (Cantarero et al., 2015), sequential reaction time (Ferrucci et al., 2013; Ehsani et al., 2016; Wessel et al., 2016) and working memory (Ferrucci et al., 2008; Pope Miall 2012). A majority of studies apply 2mA of current and place the active electrode over one cerebellar hemisphere (~3cm lateral to the inion) and the reference electrode over the ipsilateral buccinators muscle (Kim van Dun et al.,

2016). A recent modeling study has suggested that this particular montage has recently been shown to provide optimal electric field strength, while minimizing activation of other structures (Rampersad et al., 2014). Moreover, depending on the polarity applied to the cerebellar cortex, cerebellar tDCS can increase or decrease the connectivity between the cerebellum and motor cortex (i.e. modulate CBI), in the absence of motor cortex, brainstem, or spinal excitability changes (Galea et al., 2009). This finding has been interpreted to result from modulation of inhibitory output of Purkinje cells activity to the dentate nucleus, which in turn has a disynaptic connection with M1 through the thalamus. While it appears the effects of cerebellar tDCS are specific to the cerebellum, future mechanisms will need to address whether the effects of cerebellar tDCS are mediated by the Purkinje cells, cerebellar cortex or the entire cerebellum.

Scope of the Dissertation

This dissertation aims to assess and further understand the neurophysiological substrates underlying human motor learning. We investigated changes to cerebellar and M1 excitability following motor adaptation and skill learning in order to better characterize how these distinct brain regions and their physiological mechanisms contribute to learning new behaviors. The findings of the work presented here not only extend our understanding of the cerebellar and M1 role in motor learning, but also provide broad implications for developing interventions aimed at enhancing motor recovery following damage to the central nervous system. Chapter 2 and 3 are modifications of already published manuscripts (Spampinato et al., 2017; Spampinato and Celnik, 2017).

It is well known that one of the functions of the cerebellum in motor learning is to predict and account for changes imposed on the body or environment. While animal and human studies have shown that error-based driven adaptive learning leads to plastic changes within the cerebellum, the specificity of these effects in humans is unknown. In Chapter 2, we used a paired-pulse transcranial magnetic stimulation (TMS) technique to determine whether the learning-related changes in cerebellar excitability (CBI) are somatotopy-specific or reflective of a global response capable of affecting untrained effectors. We found that learning induces cerebellar excitability changes for both a trained and non-trained effector. Yet, as previously shown, we found learning transferred between these effectors and therefore the modulation of CBI found could either be related to inter-effector transfer of learning (i.e. following a somatotopy-specific plastic mechanism) or relate to a non-specific response. To disentangle this, we investigated changes in cerebellar excitability in the context of movement preparation, where no learning occurred, and found that CBI only changed for the muscle involved in movement preparation. Together, these results indicate that learning-related changes in cerebellar excitability reflect a somatotopy-

specific and further demonstrate that modulation of this physiological mechanism is also present in the context of inter-limb transfer of learning.

Distinct from error-based adaptive learning, acquiring a motor skill involves learning a de novo movement pattern where learning results in improving the trade-off between movement speed and accuracy with repeated practice. In addition to the link between error-based motor learning and cerebellar physiological changes, both animal and human studies have shown that repetitive motor learning results in LTP-like plasticity (i.e. potentiation of M1 excitability) and occlude induction of further LTP-like plasticity. In Chapter 3, we used both TMS and transcranial direct current stimulation (tDCS) to explore cerebellar and M1 physiological mechanisms when acquiring a new motor skill. We reasoned that skill learning likely engages a cerebellar dependent error-based learning to learn the dynamics of the skill task, before M1, incorporating other forms of motor learning such as reinforcement and use-dependent are engaged. We found that only early and not late skill learning modulated changes in cerebellar excitability. On the other hand, evidence of M1 LTP-like plasticity involvement occurred only during the late stages of motor skill learning. These findings highlight an important interplay between these distinct physiological mechanisms when learning a new behavior. However these results raise an important question: are the changes observed related to learning a specific component of a motor skill? Indeed, the skill learned here not only required individuals to learn how to interact with a new device/environment (sensorimotor map), but also involves performing a sequence of movements.

In Chapter 4 we addressed the question posed by the results of the previous section by deconstructing a motor skill task into its two separate motor components: sensorimotor map and specific sequence of movement patterns. We used the same physiological markers as in Chapter 3, and found that learning the sensorimotor map component resulted in modulation of cerebellar excitability, but not M1 LTP-like plasticity. Whereas, learning the sequential component elicited

cerebellar excitability changes and M1 LTP-like plasticity. These findings suggest that the nature of motor components that constitute a skill, ultimately determines which physiological mechanisms contribute to overall motor skill learning. Given our results, deficits in one brain region (M1) could be compensated by another (cerebellum).

Chapter 2 – Cerebellar-M1 connectivity changes associated to motor learning are somatotopic specific

2.1 Introduction

The cerebellum is known to play an important role in adaptive motor learning, an error-based process in which the brain learns to compensate for systematic movement errors. For example, in a visuomotor rotation, a cursor is rotated relative to hand movement such that the cursor's movement direction no longer matches the hand's. This creates a mismatch between the predicted and actual sensory outcome of a movement, which drives error reduction in healthy individuals (Tseng et al., 2007; Shadmehr et al., 2010). The cerebellum is thought to be critical for learning error-based adaptation tasks (Martin et al., 1996; Diedrichsen et al., 2005; Chen et al., 2006) since patients with cerebellar degeneration show a marked impairment in such learning (Weiner et al., 1983; Martin et al., 1996; Smith and Shadmehr, 2005). Additionally, acquisition of a visuomotor adaptation is enhanced when anodal transcranial direct current stimulation (tDCS), a form of non-invasive excitatory stimulation, is applied over the cerebellum during training (Galea et al., 2011; Block and Celnik, 2013).

While it is recognized that the cerebellum is crucial for adaptation, possible physiological mechanisms have only recently been explored (Medina and Lisberger, 2008; Carey, 2011; Schonewille et al., 2011; Gao et al., 2012; Yang and Lisberger, 2014). Transcranial magnetic stimulation (TMS) has been used to assess these neurophysiological responses in humans: a paired-pulsed TMS technique is used to stimulate the cerebellum just prior to stimulating the contralateral motor cortex (M1) (Ugawa et al., 1995; Pinto and Chen, 2001; Daskalakis et al., 2004). It is well established that dentate nucleus of the cerebellum projects to M1 (Allen and Tsukahara, 1974; Hoover and Strick, 1999), in a disynaptic excitatory pathway via the ventrolateral thalamus (Shinoda et al., 1985; Dum and Strick, 2003; Evrard and Craig, 2008).

Thus, this TMS technique is thought to measure the inhibitory projection from the cerebellar cortex to the dentate that reduces M1 activity via the dentate-thalamus-cortical pathway (cerebellar inhibition; CBI). We have recently shown that the level of CBI to a leg muscle, tibialis anterior (TA), decreases when healthy subjects learn a cerebellum-dependent locomotor adaptation task (Jayaram et al., 2011). Interestingly, those participants experiencing a larger magnitude of adaptation (i.e., greater degree of learning) expressed greater reduction in CBI. A similar reduction of CBI has also been observed in a hand muscle, first dorsal interosseous (FDI), in subjects learning a visuomotor adaptation hand task (Schlerf et al., 2012) and in response to observing or performing a finger sequence task (Torriero et al., 2011). These findings suggest a change in cerebellar excitability associated with learning. However, it is not known whether these learning-associated changes are somatotopically specific to the trained effector.

To determine whether motor learning-related changes in cerebellar-M1 connectivity are somatotopy-specific or global enough to affect untrained effectors, we examined CBI in both a hand and leg muscle (FDI and TA) before, during, and after participants learned a visuomotor rotation with the hand. Since this type learning can transfer to untrained limbs (Sainburg and Wang, 2002; Criscimagna-Hemminger et al., 2003; Wang and Sainburg, 2004; Morton and Bastian, 2006; Savin and Morton, 2008; Balitsky Thompson and Henriques, 2010; Joiner et al., 2013), modulation of CBI in an untrained limb could be related to interlimb transfer (i.e. reflecting a somatotopy-specific plastic mechanism), or it could be a non-specific response. Therefore, we also investigated CBI in the context of movement preparation, in the absence of motor learning. We predicted that modulation of CBI would be somatotopy-specific in both situations, meaning that in the motor learning task, any CBI changes in the untrained effector could be related to transfer of learning.

2.2 Materials & Methods

Subjects

In total, 32 subjects (mean age 23.9 years; 19 men) participated in three experiments (10 in Experiment 1, 10 in Experiment 2, 12 in Experiment 3). All subjects reported that they did not have conditions that would exclude them from non-invasive brain stimulation, including previous history of migraines, diabetes, seizures and any brain or peripheral nerve diseases. Exclusion criteria also included the use of nicotine, alcohol, recreational drug use and absence of prescribed medication affecting the central nervous system, all of which may alter plasticity and motor learning. All subjects reported that they were right-handed and neurologically healthy. All subjects gave informed consent approved by the Johns Hopkins Institutional Review Board.

Experiment 1: CBI changes due to visuomotor adaptation

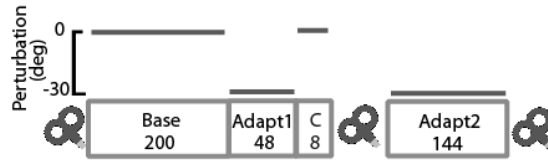
Experimental protocol. Each subject participated in an experiment testing changes in CBI in the right hand (FDI) and right leg (TA) before and during right hand visuomotor adaptation (Figure 2.1A). The experimental session consisted of five training blocks. The Baseline block consisted of 200 trials with the right hand. Following Baseline, a 30-degree clockwise perturbation (CW) was applied to the cursor display (Adapt1, 48 trials). To assess how much the individual learned, each participant underwent a quick 8-trial segment with the 30-degree clockwise perturbation turned off (Catch). Participants then completed another 144 trials with the visuomotor rotation turned back on (Adapt2) to allow them to fully correct for the perturbation. Before Baseline and immediately after Catch and Adapt2, CBI measurements were recorded for right FDI and right TA.

Behavioral tasks. Subjects were seated in front of a vertical computer screen (Figure 2.2A). A wireless digitizing pen (Wacom, Vancouver) was attached to their dominant index finger with co-flex bandage. Subjects were instructed to move their finger over a digitizing tablet (Wacom, Vancouver) positioned horizontally at waist level. Subjects viewed feedback of their finger

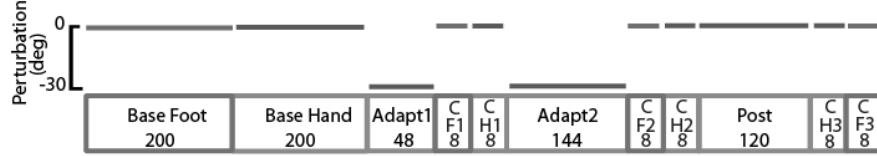
movements as a white dot (2mm) displayed on the computer screen. Subjects were instructed to move to the starting position, a white square (3mm) at the center of the screen, and then to “shoot” through one of eight targets that appeared 10 cm radially from the starting position. The 2D position of the digitizing pen was continuously recorded at 75 Hz using a custom Matlab program (Mathworks). All kinematic data were filtered at 10 Hz with a low-pass Butterworth filter and numerically differentiated to calculate velocity. The onset of each movement was determined as the point at which radial velocity crossed 5% of peak velocity. Once subjects had moved 10 cm from the starting position (10 cm circular boundary), their final position was recorded.

To ensure consistency in movement duration, subjects received auditory feedback at the end of each movement: a low-pitched or high-pitched tone if they were too slow ($>375\text{ms}$) or too fast (<275), respectively. Subjects’ vision of their hands or the digitizing tablet was blocked. Targets were displayed pseudorandomly such that every set of eight consecutive trials included one of each of the target positions. For each trial, subjects’ performance was quantified as the angular end point error, which was defined as the angle between the starting position to the center of the target and the imaginary line connecting the starting position to the end point (Hadipour-Niktarash et al., 2007; Galea et al., 2011). Epochs were created by binning 8 consecutive trials. For each block, the initial amount of error (mean error) was determined by averaging over consecutive epochs (Krakauer et al., 2005).

A. Experiment 1: CBI during visuomotor adaptation



B. Experiment 2: Assessing transfer of learning



C. Experiment 3: Measuring CBI during movement preparation

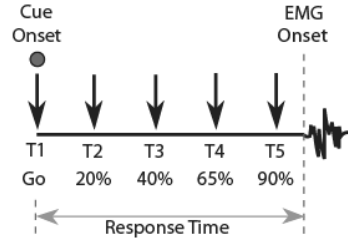


Figure 2.1: Experimental design. (A) Experiment 1 consisted of 5 behavioral blocks and three physiological measurements. In Adapt1 and Adapt2, subjects were exposed to a 30° CW cursor rotation; cursor movement was veridical in the remaining blocks. CBI was assessed before Base, and after Catch and Adapt2. The numbers in each block represent number of trials. (B) Experiment 2 consisted of 11 behavioral blocks. Adapt1 and Adapt2 had a 30° CW rotation; other blocks were veridical. Red: right foot movements. Blue: right hand movements. There were no physiological measurements for this experiment. (C) In Experiment 3, pre-movement CBI for the FDI was assessed at five different timings (T1–T5) prior to movement initiation with either the index finger or foot, with T1 at cue onset and T5 being the closest to movement onset. Timings were adjusted to individual response times. Experiment 4 mirrored the set-up of Experiment 3, but pre-movement CBI was assessed for the TA muscle.

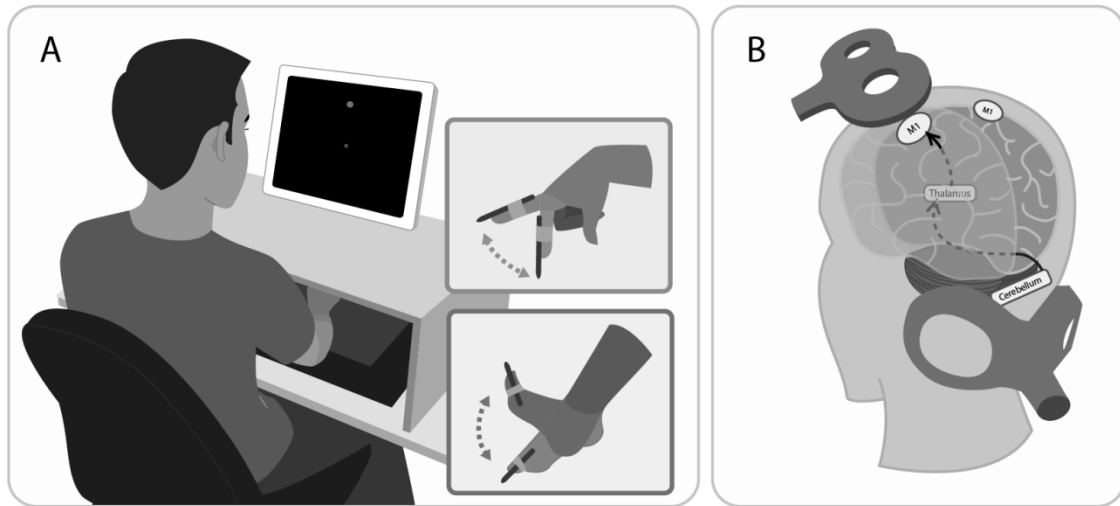


Figure 2.2: Methods. A) Set-up for the behavioral task used in all three experiments.

Participants viewed the vertical computer monitor for visual feedback about the task and trained with the right hand before switching to the right foot to assess hand-to-foot transfer.

For foot movements, the tablet was placed vertically and the foot was propped up. B) Coil

placement to measure CBI. This technique requires paired-pulse stimulation in which one TMS coil is placed over M1 (test) and the other over the cerebellum (condition). To determine CBI for the right hand muscle (FDI), the conditioning pulse was delivered over the right cerebellum 5ms before the test pulse was applied over the left M1 representation of FDI. The same procedure was followed to determine CBI for the right leg muscle (TA), with the test pulse applied over the left M1 representation of TA.

EMG Recordings and TMS protocol. Electromyography (EMG) was recorded with Ag/AgCl EMG electrodes placed over the training first dorsal interosseous (FDI) muscle and ipsilateral tibialis anterior (TA) muscle (Experiment 1: right FDI and TA, Experiment 3: right FDI). EMG data were stored for offline analysis using Signal software (CED, Cambridge). Primary motor cortex (M1) excitability of the right FDI (Experiment 1 and 3) and TA (Experiment 1 only)

muscles was assessed using a 70 mm-diameter figure-of-eight transcranial magnetic stimulation (TMS) coil (Magstim, Montreal) over the motor representation of the muscle. To localize the stimulation site and maintain consistency of responses, we used a Brain Sight neuronavigation system (Rogue Research, Montreal). After determining the resting motor thresholds (rMT) for these muscles using standard procedures (*Rossini et al 1994*), we determined the stimulator output intensity needed to elicit motor evoked potentials (MEPs) of ~1mV (SI1mV) at rest and prior to movement onset (Expt. 3 only). TMS prior to movement onset was triggered via a customized MATLAB function using the peri-triggering capability of the Signal software.

Cerebellar Inhibition (CBI). We measured the level of cerebellar-M1 connectivity or cerebellar inhibition (CBI) for right FDI and TA using a standard paired pulse TMS paradigm (Ugawa et al., 1995; Werhahn et al., 1996; Pinto and Chen, 2001; Daskalakis et al., 2004). First, we determined the brainstem motor threshold using a double cone coil (Magstim, Montreal) over the inion. This is defined as the minimal intensity (to the nearest 5% of stimulator output) required to elicit five 50 μ V MEPs of the target muscle (Rossini et al., 2015). Second, we tested CBI by delivering a conditioning stimulus (CS) 3cm lateral to the inion and 5ms prior to a test stimulus (TS) targeting the contralateral M1 representation of FDI or TA (Galea et al., 2009; Jayaram et al., 2011; Schlerf et al., 2015).

CS intensity was set at 5% below the brainstem motor threshold to the cerebellum. This elicits maximum recruitment of the cerebellar-M1 connections, whereas lower intensities results in less inhibition of this pathway (Werhahn et al., 1996; Pinto and Chen, 2001; Daskalakis et al., 2004; Galea et al., 2009). Test stimulus over M1 was adjusted to elicit MEPs with average peak-to-peak amplitude of ~1 mV (SI1mV), which is ideal for CBI assessment (Ugawa et al., 1995; Pinto and Chen, 2001; Daskalakis et al., 2004). Ten paired pulses (cerebellum + M1) and 10 single pulses (M1 only) were randomly intermixed and delivered at 5s intervals. CBI is expressed as the

average MEP amplitude evoked by the cerebellar-conditioned stimulation relative to the average MEP amplitude evoked by the unconditioned TMS pulses over M1.

Experiment 2: Transfer from right hand to right leg

Experimental protocol. Each subject completed 11 behavioral blocks. No physiological measurements were tested in this experiment. (Figure 2.1 B). 200 trials with the right foot (Baseline Foot) were followed by 200 trials with the right hand (Baseline Hand). As in Experiment 1, a screen-cursor 30-degree CW transformation was applied during Adapt1 and Adapt2 (48 trials each, right hand) to elicit adaptive learning. Immediately after Adapt1, the transformation was removed and 8 trials in the foot (Catch Foot1) and hand (Catch Hand1) were performed to determine the presence of transfer of learning to the foot. Transfer was again assessed after Adapt2 with another non-perturbed 8 trials with the foot and hand (Catch Foot2 and Catch Hand2). Participants then completed a final 120 hand movements without perturbation to washout the learning (Post). Finally, another 8 trials with the foot and hand were assessed to see if any previously seen transfer would washout as well (Catch Foot3 and Catch Hand3). No physiological measurements were made.

Behavioral tasks. Participants were seated in front of a computer screen as in Experiment 1. In addition, a bench was placed between the computer station and chair. The right leg was supported comfortably such that the foot was lifted a few inches off the floor, with the leg remaining parallel to the floor. For experimental blocks using the leg, the digitizing tablet was clamped vertically in front of the right foot. The digitizing pen was attached with co-flex as an extension to the inner side of the bare foot. Feedback (both visual and auditory) of foot movements was the same as described for hand movements. To reduce the complexity of the performance with the foot, we presented only 4 targets (up, down, left and right from the center) out of the possible 8; the order of appearance of these targets was random. The hand was exposed to the 8 different target locations as described in Experiment 1.

Experiment 3&4: Pre-movement CBI changes due to simple reaction time task

Experimental protocol. Each subject participated in two sessions testing CBI in the right hand (FDI; Experiment 3) or the right leg (TA; Experiment 4) during a simple reaction time task. In Experiment 3 only, a CBI recruitment curve for the FDI muscle was assessed at rest. In Experiment 4 we used a double-cone coil over M1 in order to elicit MEPs at rest for the TA muscle. For each Experiment, subjects performed abduction of the right index finger in one session and dorsiflexion of the right foot in the other session, with session order counterbalanced across subjects. Paired- and single-pulse TMS was applied at intervals during movement preparation.

Behavioral tasks. Subjects were seated in front of a computer monitor and were instructed to respond to a visual (green circle) go signal by lifting either the right index finger or lifting the right foot, in separate sessions. Go signal appeared at random intervals (5–7 seconds). Prior to the appearance of the go signal, subjects were instructed to remain relaxed and avoid anticipation of the cue. Response time (RT) was defined as the interval between the go signal and the onset of the EMG burst in FDI or TA (Figure 2.1C). At the beginning of each session, subjects were familiarized with the simple reaction time paradigm and 30 trials were performed to characterize each subject's individual response time (RT) to the go signal in the absence of transcranial magnetic stimulation (TMS). A total of 120 trials were completed per session. Trials where background EMG was detected prior to TMS onset were excluded from analysis.

EMG recordings and TMS protocol were identical to those in Experiment 1, with the following exceptions: EMG was recorded from either for the right FDI in Experiment 3 or the right TA in Experiment 4, and TMS measures were made for the M1 representation of right FDI (Experiment 3) or right TA (Experiment 4). In addition, we determined the stimulator output intensity needed to elicit MEPs of ~1mV (SI1mV) both at rest and prior to movement onset. TMS prior to

movement onset was triggered via a customized MATLAB function using the peri-triggering capability of the Signal software.

Cerebellar-M1 connectivity (CBI) protocol was identical to that in Experiment 1, except we measured the level of cerebellar-M1 connectivity (CBI) for right FDI in Experiment 3 and for the right TA in Experiment 4. In addition, CS intensity for Experiment 3 was based on a CBI recruitment curve (RC_{CBI}). In Experiment 4, the CS intensity was set at 5% below the brainstem motor threshold to the cerebellum. This intensity was selected since cerebellar-M1 connections for the TA muscle show reduced inhibition in comparison to the FDI muscle (Jayaram et al., 2011).

We computed RC_{CBI} at rest prior to beginning the Experiment 3 behavioral session. This was done by decreasing cerebellar CS intensity by -5% steps below brainstem threshold using four different CS intensities (-5, -10, -15, and -20% brainstem threshold). For each subject, the CS intensity inflection point collected from RC_{CBI} was used for pre-movement CBI measurements (CBI_{move}) to evaluate whether movement preparation elicits further inhibition or dis-inhibition.

For both Experiment 3 and 4, CBI_{move} was measured at five different time intervals throughout the course of the simple reaction time paradigm in pseudo-randomized order (Figure 2.1C; T1–T5). To do this, 12 paired pulses (cerebellum + M1) and 12 single pulses (M1 only) were measured for each time interval. As described previously, the different time intervals were tuned to each subject's RT of the task (Murase et al., 2004; Duque et al., 2005; Hummel et al., 2009), where T1 corresponded to cue onset and T2-T5 reflects 20, 40, 65, and 90% of subject RT respectively.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, IL). For each block in Experiment 1 and 2, apart from catch trial

blocks, initial error (mean error) was determined by averaging across epochs (Krakauer 2005). Repeated measures analysis of variance (ANOVA_{RM}) was used to compare changes in CBI across time points (Baseline, Adapt1, Adapt2) and muscles (Right FDI vs. Right TA), and separately to compare mean movement error across time points (Baseline and Catch epochs) and limbs (hand vs. foot in Experiment 2).

In Experiment 3&4, we averaged each participant's RTs in bins of 30 trials and used a paired sample t-test to compare the first 30 RT trials to the last 30 trials to determine if participants were improving their performance throughout the task. We expressed the magnitude of CBI_{move} at T1-T5 relative to CBI recorded at rest (e.g., CBI_{move} T5 modulation = (CBI at T5)/(CBI at rest) × 100%) to better characterize changes in CBI relative to rest. Subsequently, to compare changes in CBI_{move} across sessions (finger-movements, foot-movements), we used ANOVA_{RM} with pre-movement timing (T1-T5) as a within-subjects factor. For Experiment 3, we also used ANOVA_{RM} to compare the effect of changing CS intensity (-5, -10, -15, and -20% below brainstem threshold) on CBI (RC_{CBI}). For all experiments, when ANOVAs yielded significant results, post-hoc analyses were conducted using Tukey HSD tests.

2.3 Results

Experiment 1: CBI decreases after visuomotor adaptation

When the 30 degree cw visuomotor transformation was applied in Adapt1, large initial errors were observed (first epoch mean ± SE was $-26.45 \pm 2.24^\circ$; Figure 2.3A). When the visuomotor transformation was removed for a Catch epoch after 48 movements, subjects displayed partial learning of the rotation, as indicated by ccw errors ($11.08 \pm 0.97^\circ$). When the perturbation resumed at the beginning of Adapt2, error reduction continued and by the end of Adapt2, subjects had compensated for $25.92 \pm 1.03^\circ$ of the original 30° perturbation.

The magnitude of CBI for both the FDI and TA muscles (hand and leg) was reduced after adaptation with the right hand, indicating disinhibition (Figure 2.3B). An ANOVA_{RM} on CBI ratio values revealed a time effect ($F(2,36) = 9.785$; $p < 0.01$) but no effect on muscle group ($F(1,18) = 2.74$; $p = 0.12$) and importantly, no interaction ($F(2,36) = 0.16$; $p = 0.90$). Post-hoc tests revealed that CBI after Catch and after Adapt2 was significantly different from baseline CBI ($p < 0.01$; $p = 0.01$, respectively), suggesting that visuo-motor adaptation with the hand results in a significant reduction in CBI for both FDI and TA muscles.

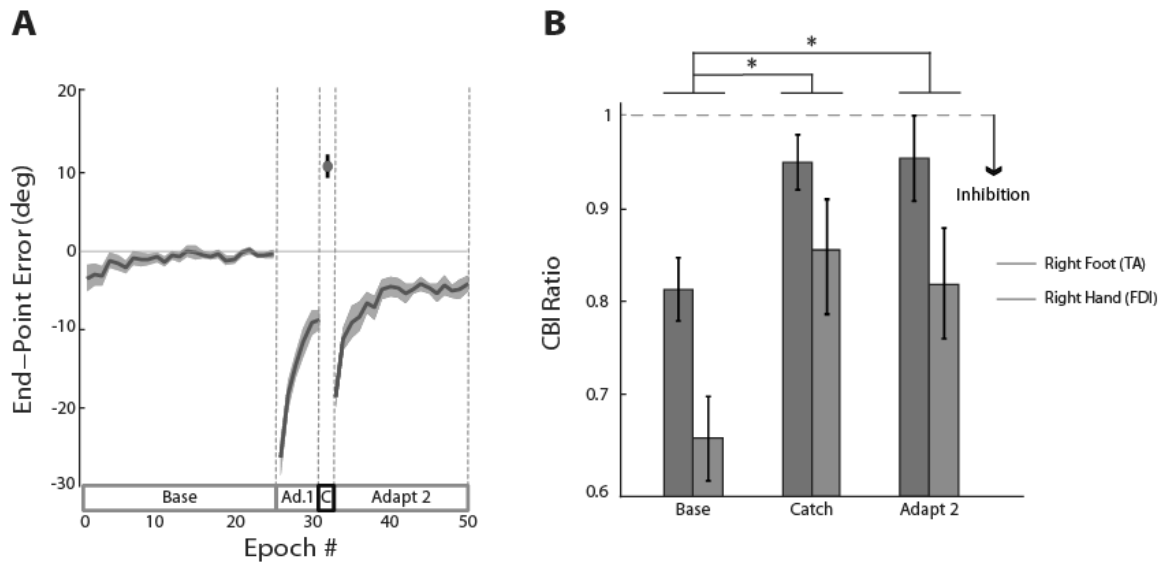


Figure 2.3: Experiment 1 Results. (A) End point error (blue line) with standard errors (shaded region) during baseline (Base), adaptation (Ad.1 and Adapt2), and catch trials (C). Negative values indicate clockwise deviations caused by the visuomotor perturbation. (B) Physiological measure of CBI for both the right FDI (blue) and TA (red). CBI was recorded before any movements (Base), and immediately after catch trials (Catch) and late adaptation (Adapt2). * CBI decreased significantly for both muscle effectors after early perturbation exposure.

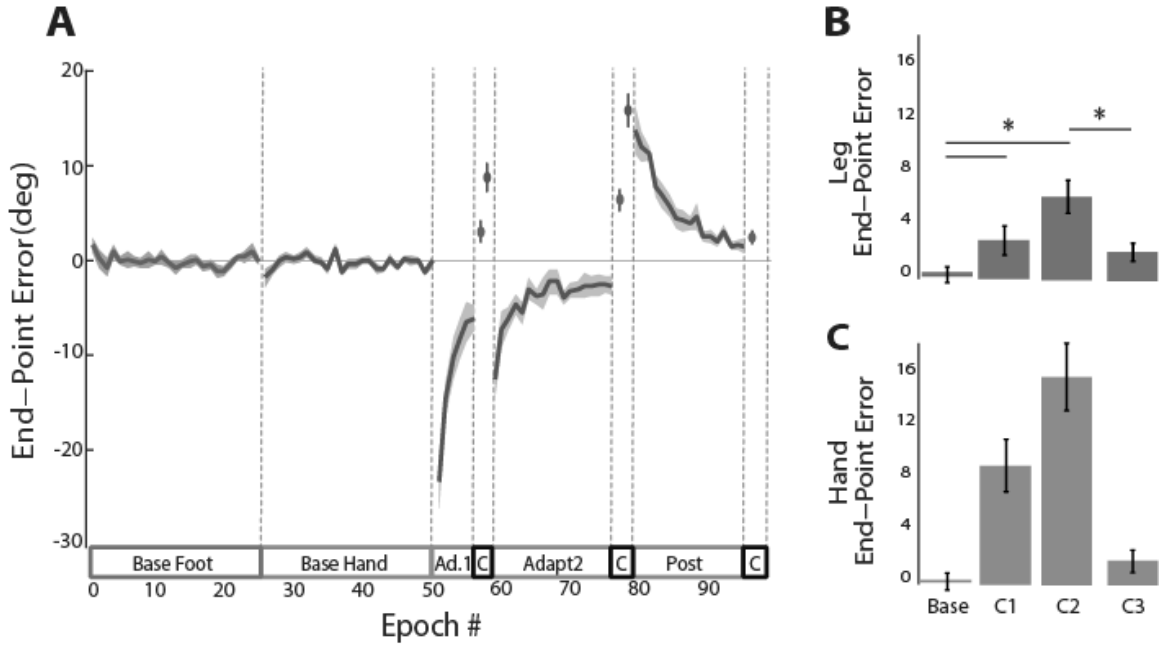


Figure 2.4: Experiment 2 Results. (A) End point error and standard errors for right hand (blue) and right leg (red) movements. Negative values indicate clockwise deviation. (B-C) Mean end-point errors in degrees (\pm SEM) for right leg (red) and right hand (blue) for the baseline and three catch trial epochs. Post-Hoc analysis revealed significant changes in error for both effectors, indicating hand-to-foot transfer.

Experiment 2: Right hand learning transfers to right foot movements

As in Experiment 1, subjects in Experiment 2 were able to almost completely adjust their hand movements to the 30° cw visuomotor rotation (mean error of final epoch in Adapt2 was $-2.32 \pm 1.11^\circ$, Figure 2.4). When comparing behavioral blocks across right hand and right leg (Baseline, Catch1, Catch2, and Catch3), ANOVA_{RM} revealed a significant effect of time ($F(2,36) = 24.94$; $p < 0.01$), limb ($F(1,36) = 9.44$, $p = 0.01$), and limb x time interaction effect ($F(2,36) = 5.63$; $p < 0.01$). Post-hoc analysis revealed that movement error in Catch Foot1 and Catch Foot2 were each

different from Base Foot ($p = 0.05, 0.01$), suggesting that the right hand's adaptation transferred to the foot. Critically, Post-hoc analysis also revealed a difference in Catch Hand1 and Catch Hand2 being different from Base Hand (both $p < 0.01$). A comparison of foot aftereffects (Catch Foot 1-2) to hand aftereffects (Catch Hand 1 and the first epoch of Post 1) indicated that the amount of transfer at Foot Catch1 was 42.3%, and 42.2% at Foot Catch2. Post hoc tests also showed that Catch Foot2 and Catch Foot3 were different from each other ($p < 0.01$), suggesting that transfer of learning from hand to foot had degraded after washout of learning from the hand.

Experiment 3 & 4: CBI changes in a somatotopic specific manner

Reaction Times. Participants did not improve their RT throughout the experiment. For Experiment 3 the baseline average RT for the hand (176.1 ± 6.2 ms) was not significantly different from RT from the last 30 hand trials (174.0 ± 5.6 ms; $t(11) = 1.242, p = 0.24$). Baseline foot RT (191.9 ± 8.7 ms) was also not different from the last 30 foot trials (190.3 ± 7.5 ms; $t(11) = 0.8, p = 0.44$). Similarly in Experiment 4, baseline foot RT (196.0 ± 5.7 ms) was not different from the last 30 foot trials (193.3 ± 5.4 ms; $t(7) = 0.971, p = 0.36$), and baseline hand RT (181.2 ± 7.3 ms) was not different from the last 30 hand trials (179.9 ± 6.7 ms; $t(7) = 0.759, p = 0.47$).

CBI Recruitment Curve (Experiment 3 only). ANOVA_{RM} comparing the CBI ratio across CS intensity revealed a significant effect for CS intensity ($F(1,11) = 7.926, p < 0.01$). Critically, TS MEP amplitudes were not significantly different across different CS conditions ($F(1,11) = 0.029, p = 0.87$), suggesting the CBI changes were due to changing CS intensities. As a result, the mean CS intensity set for CBI_{move} was $72.9 \pm 0.74\%$ of the stimulator output, yielding a rest CBI ratio response of 0.82 ± 0.07 .

Pre-movement CBI_{move}. In order to assess whether CBI changes are somatotopy-specific, we measured pre-movement CBI (CBI_{move}) for the FDI (Experiment 3) and TA (Experiment 4) muscle representation when participants were asked to either move the right index finger or right foot. In Experiment 3, when participants made movements with their finger, ANOVA_{RM} revealed an effect of FDI CBI_{move} for time (response time) ($F(4,44) = 8.192, p = 0.02$; Figure 2.5A). On the other hand, when FDI CBI_{move} was recorded in preparation of foot movements, ANOVA_{RM} failed to find the same effect ($F(4,44) = 3.214, p = 0.22$; Figure 2.5B). These results indicate that in the absence of learning, CBI changes of the FDI muscle occurs during the preparation of finger movements, but not the foot. In Experiment 4, when participants made movements with the right foot, ANOVA_{RM} on TA CBI_{move} values revealed a time effect ($F(4,28) = 4.920, p < 0.01$; Figure 2.5D). Conversely, when TA CBI_{move} was recorded in preparation of finger movements, ANOVA_{RM} did not reveal the same effect ($F(4,28) = 0.241, p > 0.50$; Figure 2.5E). Together the results from Experiment 3 & 4 suggest a somatotopic effect of CBI changes during movement preparation.

Furthermore, to directly compare the changes in FDI CBI_{move} between preparation of finger movements and foot movements, we subtracted the amount of CBI measured at rest for each session. Here, ANOVA_{RM} revealed an effect of FDI CBI_{move} for group (finger, foot; $F(1,22) = 6.214, p = 0.02$) and response time (Go, T1,...,T5) x group interaction ($F(4,88) = 4.713, p = 0.01$; Figure 2.5C). Specifically, CBI_{move} recorded during movement preparation at 90% response time (T5) was significantly different from CBI assessed at cue representation ($p = 0.03$), indicating that specific FDI CBI changes occur just prior to movement onset. We performed the same analysis to compare the changes in TA CBI_{move}. Similar to the results of Experiment 3, ANOVA_{RM} showed an effect of TA CBI_{move} for group (foot, finger; $F(1,14) = 7.911, p = 0.02$) and response time (Go, T1,...,T5) x group interaction ($F(4,56) = 3.210, p = 0.02$; Figure 2.5F).

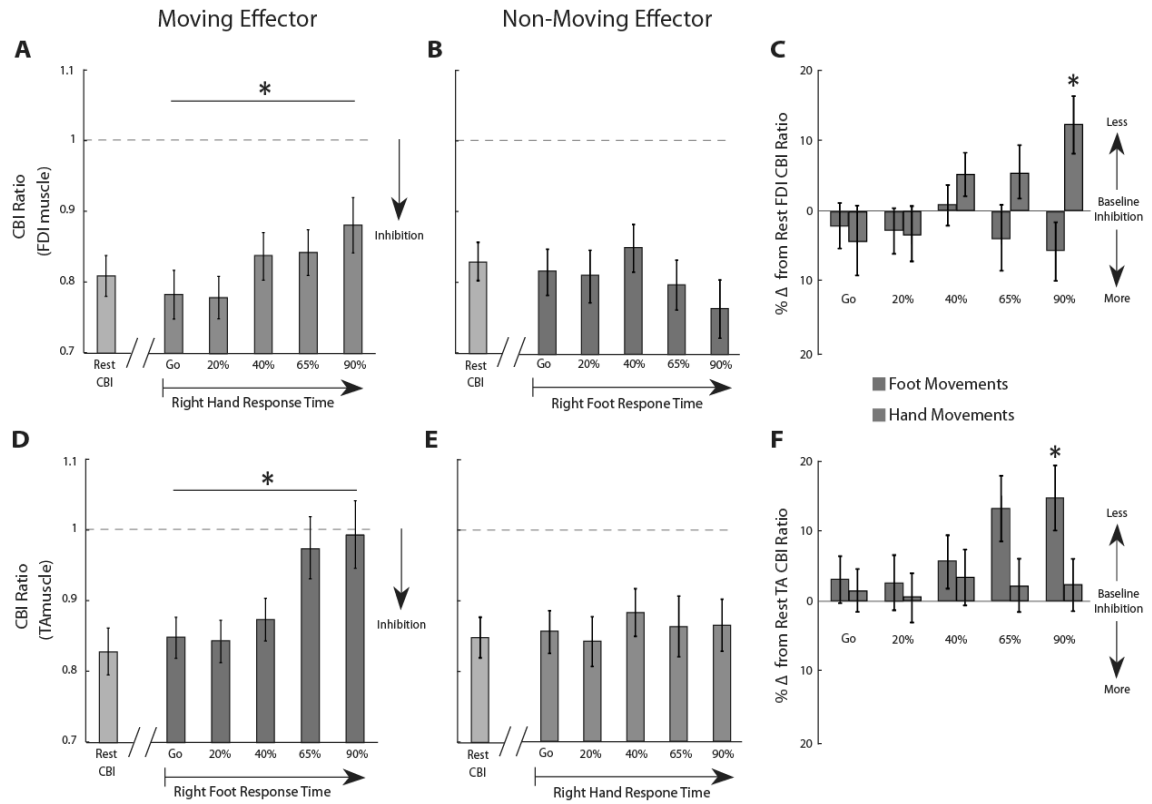


Figure 2.5: CBI in preparation of movements (CBI_{move}). (A-B) The x-axis represents CBI_{move} for the right FDI in preparation to moving the hand (A) and foot (B). FDI CBI_{move} was measured at five timings (T1–T5) with respect to individual mean reaction times separately for the hand (blue) and foot (red). *CBI was reduced significantly only in preparation of hand movements. (C) We calculated CBI_{move} as % difference from FDI CBI obtained at rest. (D-E) The x-axis represents CBI_{move} for the right TA in preparation to moving the foot (D) and foot (E). (F) We calculated the % difference from TA CBI obtained at rest. Positive values indicate disinhibition and negative values increased inhibition. *Only hand movements at 90% response time (T5) modulated CBI_{move} . Data are mean \pm SEM.

Importantly, we determined that modulation of CBI were due to changes in conditioned (CS+TS) responses from the cerebellum and not due to changes in test stimulus (TS) responses from M1. Although ANOVA_{RM} revealed a significant effect of TS MEP amplitude during finger movement preparation (Go, T1,...,T5; $F(4,44) = 14.823, p = 0.001$), we controlled for this confound by measuring FDI CBI at rest with a matched TS MEP amplitude observed at the 90% response time of CBI_{move} (TS ~2mV; Figure 2.6). When we compared TS and CS+TS MEP amplitudes between rest and pre-movement measurements using matched TS responses, two-way ANOVA_{RM} showed a significant interaction of MEP amplitudes for state (rest, pre-movement) x condition (TS, CS+TS; $F(1,22) = 6.295, p = 0.043$). Specifically, CS+TS MEP amplitude for pre-movement was different from rest ($p = 0.02$), despite having comparable TS MEP amplitude ($p = 0.458$). This indicates that the changes in FDI CBI_{move} observed at 90% response time are due to changes in cerebellar excitability (CS+TS) and not due to higher M1 excitability.

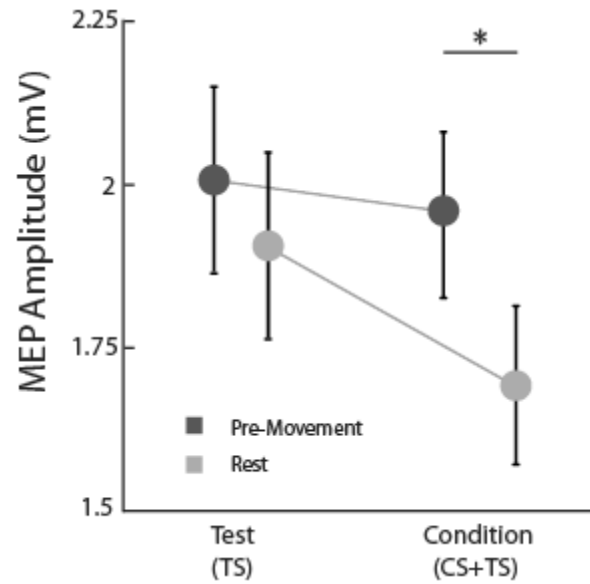


Figure 2.6: Rest and Pre-Movement Test Stimulus (TS) and Conditioned Stimulus (CS+TS) MEP Amplitudes. For each participant, we assessed CBI at rest matching the TS MEP amplitudes obtained during CBI_{move} at 90% of response time (TS). CS+TS MEP amplitude (CBI) was only present at rest (green), but not when assessed in the context of movement (purple). This indicates that the reduction of CBI during movement (CBI_{move}) is not due to increased excitability in M1.

2.4 Discussion

Here we address the question of whether changes in cerebellar-M1 connectivity (CBI) are somatotopy-specific in the presence or absence of adaptive motor learning. When individuals learned a visuomotor rotation with the hand, CBI changed for both the involved hand and idle ipsilateral foot. The transfer of learning we observed between hand and foot could explain why CBI changed in both representations. We disentangled this issue by measuring CBI in the context of movement preparation of a well-characterized behavior, with no learning. We found that CBI for the hand only changed when participants prepared to make hand movements and not foot movements. Similarly, CBI measured for the foot only changed with preparation of foot movements. This indicates that in the absence of learning, and transfer of learning, CBI only modulates for the effector involved in the movement. These results address an important physiological question, showing that modulation of cerebellar-M1 connectivity responses reflect a somatotopy-specific mechanism.

We have previously found CBI changes for the trained hand in a visuomotor adaptation task (Schlerf et al., 2012), as well as for the trained leg during locomotor adaptation (Jayaram et al., 2011). The present study extends these findings by showing that adaptive learning with the hand can produce changes in CBI for muscle representations not involved in the task. Interestingly, our second experiment showed transfer of learned movements experienced with the right hand to the right foot, which we would expect to cause CBI changes in both representations. Thus the non-specific changes in cerebellar excitability observed in our first experiment are likely related to a transfer of learning from hand to leg. Results from our final two experiments show that in the absence of learning, CBI modulates specifically for the muscle involved in movement preparation. Taken together, these findings indicate that cerebellar-M1 connectivity changes are somatotopy-specific.

We observed CBI changes during both movement preparation and after adaptive learning, but interpret these results to be driven by different mechanisms; premovement CBI is likely due to activity patterns of Purkinje cells (PCs) and deep cerebellar nuclei (DCN), whereas learning-induced changes in CBI reflect cerebellar plastic changes. Although the physiology of cerebellar TMS remains poorly understood, it is likely possible that stimulation results in the parallel fiber-mediated activation of PCs, which inhibit the DCN (Celnik 2015). The reduced CBI closer to movement initiation in this study may therefore represent a decrease of inhibition from hand- or leg-affiliated PCs that activate hand- or leg-affiliated DN cells, respectively. Animal studies have shown burst activity of DCN during preparation of limb movement, where inactivation of the cerebellum results in delay of M1 activity and delay in the initiation of movements (Brooks, 1975; Miller and Brooks, 1982). In addition, suppression of opto-genetically modified PCs in rodents can activate dentate cells (Heiney et al., 2014), suggesting that the onset of activity in DCNs results from disinhibition by PCs. Furthermore, a recent study in monkeys showed that prior to wrist movement onset, wrist-affiliated PCs were suppressed while wrist-affiliated DN cells showed concurrent burst activity without prior suppression (Ishikawa et al., 2014). Thus during the preparation of a specific muscle movement, the cerebellar cortex may reduce its inhibition to M1 via the cerebello-thalamo-M1 pathway, consistent with the results found in this study.

In contrast, we interpret the CBI changes in learning to reflect the plastic changes in cerebellar output that are responsible for changing motor behavior during adaptation. Although the cerebellum contains multiple sites and forms of plasticity (Boyden et al., 2004; Jorntell and Hansel, 2006; Gao et al., 2012), two plasticity sites have been shown to be important for motor learning: (1) long-term potentiation (LTP) of mossy fibers and interneurons in the cerebellar cortex (D'Angelo, 2005; Grasselli and Hansel, 2014) and (2) long-term depression (LTD) of parallel fiber –PCs synapses. In particular, animal studies have associated LTD in PCs with

adaptive learning, triggered by climbing fiber inputs driven by inaccurate movements (Gilbert and Thach, 1977; Medina and Lisberger, 2008; Yang and Lisberger, 2014). Since errors are prevalent early in adaptive learning, we interpret our CBI changes to reflect reduced PCs activity. Accordingly, if PCs are less excitable, then a conditioning stimulus would be less likely to engage the cerebellar-dentate-thalamic pathway, which would result in less M1 inhibition, consistent with the results of this study. However, it is important to consider that LTP of parallel fibers and inhibitory interneurons can result in the same net effect as LTD of parallel fiber-PCs synapses (D'Angelo, 2014; Jorntell, 2016). Thus, it is possible that multiple plasticity mechanisms throughout the cerebellum operate in learning a new behavior (Medina and Mauk, 2000; Jorntell and Ekerot, 2003; Yang and Lisberger, 2014; Mapelli et al., 2015).

The novel finding of this study is that learning-related changes in CBI are somatotopic specific. As described previously (Morton and Bastian, 2004; Savin and Morton, 2008), we found that adaptive learning transfers between the arm and leg. In addition to this behavioral finding, we show a similar effect using a cerebellar physiological measure. Thus, the transfer in CBI changes found in Experiment 2 appears related to the transfer of learning, since CBI follows a somatotopic specific pattern when assessed in the context of movement preparation.

Several neuroimaging studies have demonstrated gross motor somatotopy for upper and lower limb representations within the cerebellar cortex (Nitschke et al., 1996; Rijntjes et al., 1999; Grodd et al., 2001; Stoodley and Schmahmann, 2009; Schlerf et al., 2010; Buckner et al., 2011) and the dentate nucleus (Dimitrova et al., 2006; Kuper et al., 2012). In addition, studies in non-human primates have reported that dentate nucleus somatotopic representation of the lower limb is located more rostrally compared to the upper limb (Allen et al., 1978; Rispal-Adel et al., 1982; van Kan et al., 1994). Indeed, retrograde axonal transport of neurotropic viruses injected in

different body representations of M1 demonstrated a rostral-caudal output organization of leg, arm and face representations in the dentate nucleus (Dum and Strick, 2003; Lu et al., 2007). However, there is also evidence of anatomical overlap for these representations. For example, electrical stimulation of the cerebellar nuclei can cause concurrent movement of lower and upper limbs (Rispa-Padel et al., 1982), and in some cases, dentate neurons reacted to both lower and upper-limb movements (van Kan et al., 1994). In humans, fMRI studies have suggested that there is extensive overlap between finger and foot movement activation when looking at group analysis of cerebellar cortex (Rijntjes et al., 1999) and dentate nucleus activation (Kuper et al., 2012).

It is not known to what extent the overlapping arm and leg representations can interact with each other, but the microarchitecture of the cerebellar cortex raises the possibility that such interactions may occur within the cerebellum and serve as a substrate of ipsilateral transfer. A single mossy fiber may have synaptic contacts with 448 cerebellar granule cells (Itō, 1984), and the parallel fibers of each granule cell branch and excite hundreds of Purkinje cells up to several millimeters from the branch point (Fox and Barnard, 1957). Climbing fibers also branch, and although each Purkinje cell receives only one climbing fiber input, each climbing fiber may synapse with 10 Purkinje cells (Eccles et al., 1966). Thus it is possible that some Purkinje cells that stimulate the leg representation receive both parallel fiber and climbing fiber inputs from the arm and are therefore able to undergo plastic modifications in response to visuomotor learning with the ipsilateral arm, leading to transfer. An alternative possibility is that within a cerebellar hemisphere, arm and leg representations interact with each other through the large network of inhibitory interneurons present throughout cerebellar cortex (Itō, 1984). For example, a single Golgi cell receives ~228 mossy fiber and ~4788 parallel fiber inputs, in addition to inputs from climbing fibers, Purkinje collaterals, and other interneurons, and inhibits up to 5700 granule cells (Itō, 1984). Golgi cells and other cerebellar interneurons are thought to be involved in the specificity and modulation of cerebellar cortical computations (Itō, 1984).

In addition to demonstrating that cerebellar-M1 connectivity is organized somatotopically, we describe a novel method to measure cerebellar-M1 connectivity physiology during movement preparation. This is important because previous investigations have only provided evidence that the cerebellum exerts an influence on M1 at rest or in an indirect manner. For example, pre-movement facilitation of MEPs normally observed in response to M1 TMS is reduced in patients with spino-cerebellar degeneration (Nomura et al., 2001) and unilateral cerebellar stroke (Battaglia et al., 2006); which has been linked with deficits in motor preparation and motor imagery in these populations. Thus, the paradigm used in this study could be employed in future research to assess context-dependent (pre-movement) physiological changes in patients to further understand the role of the cerebellum in movement preparation. Furthermore, future investigations will need to address what aspects of behavioral transfer are associated with changes in CB-M1 connectivity.

Chapter 3 – Temporal dynamics of cerebellar and motor cortex physiological processes during motor skill learning

3.1 Introduction

The ability to acquire and retain motor skills is critical to the animal kingdom. Here, we refer to motor skills as the ability to improve movement speed and accuracy with repeated practice. For instance, a novice tennis player first serves tennis balls at lower speeds and with limited accuracy, but with training is able to hit high-speed balls with improved accuracy. This skill improvement likely involves multiple stages, including an early component where rapid within-session improvements are observed and are likely a result of rapid acquisition of task dynamics (i.e. tennis racquet weight, ball conditions, etc.), and a second slower phase where memories are stored and readily available for retrieval (Dayan and Cohen, 2011; Penhune and Steele, 2012).

Several studies including functional MRI, non-invasive brain stimulation studies (NIBS) and behavioral investigations in patients with cerebellar diseases have indicated a critical role of the cerebellum (CB) earlier on during motor learning (Martin et al., 1996; Smith and Shadmehr, 2005; Morton and Bastian, 2006; Tseng et al., 2007; Galea et al., 2011; Jayaram et al., 2011; Schlerf et al., 2012; Cantarero et al., 2015). Some of these investigations also showed that after an initial increase in cerebellar activity there is a decrease of activation over time (Floyer-Lea and Matthew, 2005; Lehericy et al., 2005; Seidler 2006; Steele and Penhune, 2010). However, other studies also implicated the CB in retention or later phases of learning (Imamizu et al., 2000, 2003; Graydon et al., 2005; Luaute et al., 2009; Wessel et al., 2016; Kim et al., 2015)

Activity in the primary motor cortex (M1) has also been critically implicated during motor learning. While some studies showed increased activation with motor practice (Karni et al., 1995; Penhune and Doyon, 2005; Steele and Penhune, 2010), others described decreasing activity with

training (Wu et al., 2004; Seidler et al., 2005). Similarly, behavioral and NIBS research has indicated that the CB is critically involved during the acquisition of motor tasks (Smith and Shadmehr, 2005; Galea et al., 2011; Cantarero et al., 2015; Donchin et al., 2012), whereas M1 is involved in the encoding of learned movements (Galea et al., 2011; Muellbacher et al., 2001; Reis et al., 2009; Robertson et al., 2004; Richardson et al., 2006), suggesting that distinct stages of skill learning weight the cerebellar and M1 roles differently. Part of the inconsistencies across investigations might result from different studies testing different motor tasks with different techniques. Therefore, the specific temporal contributions of the CB and M1 during different stages of skill learning remains incompletely understood.

The CB is thought to contribute to motor learning by predicting and accounting for systematic changes to the body or the environment, resulting in the correction of errors on a trial-by-trial basis. Animal work has shown that this form of adaptive learning is mediated, in part, by long-term depression of parallel fiber-Purkinje cell synapse in cerebellar cortex (Medina and Lisberger, 2008; Yang and Lisberger, 2014). In humans, studies using transcranial magnetic stimulation (TMS) to assess the inhibitory tone the CB exerts over M1 (cerebellar inhibition, CBI) have described changes in cerebellar excitability during motor adaptation studies (Galea et al., 2011; Jayaram et al., 2011). On the other hand, both animal and human research have shown that motor learning elicits long-term potentiation (LTP) changes in M1, resulting in a reduced capacity to induce more LTP-like changes, a phenomenon known as occlusion (Nudo et al., 1996; Kleim et al., 1998, 2002; Rioult-Pedotti et al., 1998, 2000, 2007; Ziemann et al., 2004; Rosenkranz et al., 2007; Lepage et al., 2012; Avanzino et al., 2015). Evidence for occlusion of M1 LTP-like plasticity immediately after skill learning can be assessed by applying anodal transcranial direct current stimulation (Anodal tDCS) combined with TMS (Cantarero et al., 2013 a&b). While changes in cerebellar excitability and occlusion of M1-plasticity represent physiological

markers of cerebellar and M1 contributions to learning, these mechanisms have never been directly tested on the same motor skill task.

Here we sought to assess neurophysiological mechanisms in the CB and M1 of humans during early and late skill learning using TMS and AtDCS to understand the temporal contributions of cerebellar and M1 networks during skill learning. We hypothesized that CBI will change early during skill learning, whereas occlusion of LTP-like plasticity in M1 will be more prominent later on as the skill is sufficiently practiced and stored. Importantly, to understand the specificity of these markers, we also assess other measures of cortical and intracortical excitability.

3.2 Results

All participants trained for two consecutive days on the sequential visual isometric pinch task (SVIPT), where squeezing a force transducer with the right thumb and index finger controls the movement of an on-screen computer cursor. We randomly assigned participants to one of three distinct behavioral groups: Long ($n=10$), Short ($n=11$) or Random ($n=8$). On each day, the Long and Random groups completed 150 trials (5 blocks; 1 block=30 trials) of the SVIPT, whereas the Short Training Group only completed 1 block of 30 trials (Figure 3.1). In addition to motor training, all participants underwent physiological measurements to assess changes in CBI, M1 LTP-like capacity (occlusion) and corticomotor excitability (s1Mv, SICI; see Methods below for description of each neurophysiological measurement). For the Long and Random groups, we assessed CBI, s1mV, and SICI before training was initiated, as well as after behavioral blocks 1, 3, 5 (Figure 3.1, Pre-P3) on each training session (Day1, Day2). We recorded all physiological measures for the Short group only prior to and after completion of one behavioral block (i.e. early stage of learning). For each group, we measured AtDCS induced M1-LTP-like plasticity aftereffects at rest (Day 0) and after completion of each training session (Day1, Day2).

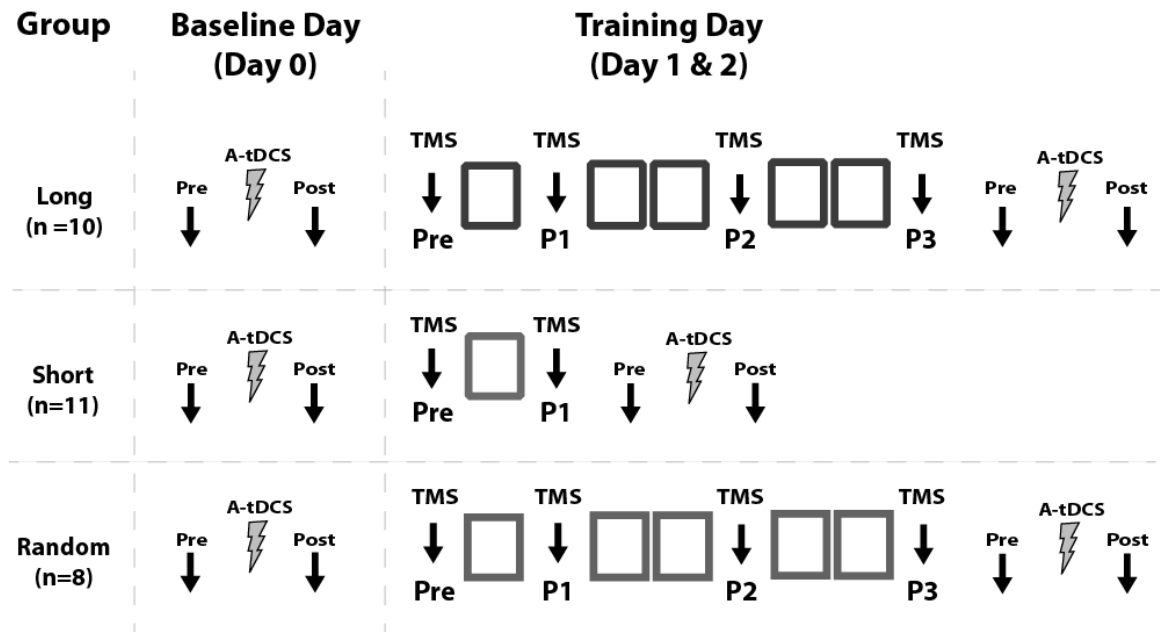


Figure 3.1: Experimental design for all groups. Long group (n=10) individuals participated in two days of motor skill training (Day1; Day2), completing five blocks of 30 trials each day (Day1 and Day2). TMS measurements (black arrows) for this group was assessed prior to training (Pre) and after the first (P1), third (P2), and final behavioral block (P3). On each training session, individuals in the short group (n=11) trained only on one block of the skill task and TMS physiological assessments for these individuals were recorded prior to and after completion of 1 block (Pre; P1). Random group (n=8) was identical to the Long group, except that Random group individuals performed in a randomized version of the task. In all groups, MEP amplitudes (black arrows) were measured before and after application of A-tDCS (grey ray). This was assessed on separate days, when there was no training (Day0) and after training (Day1 and Day2).

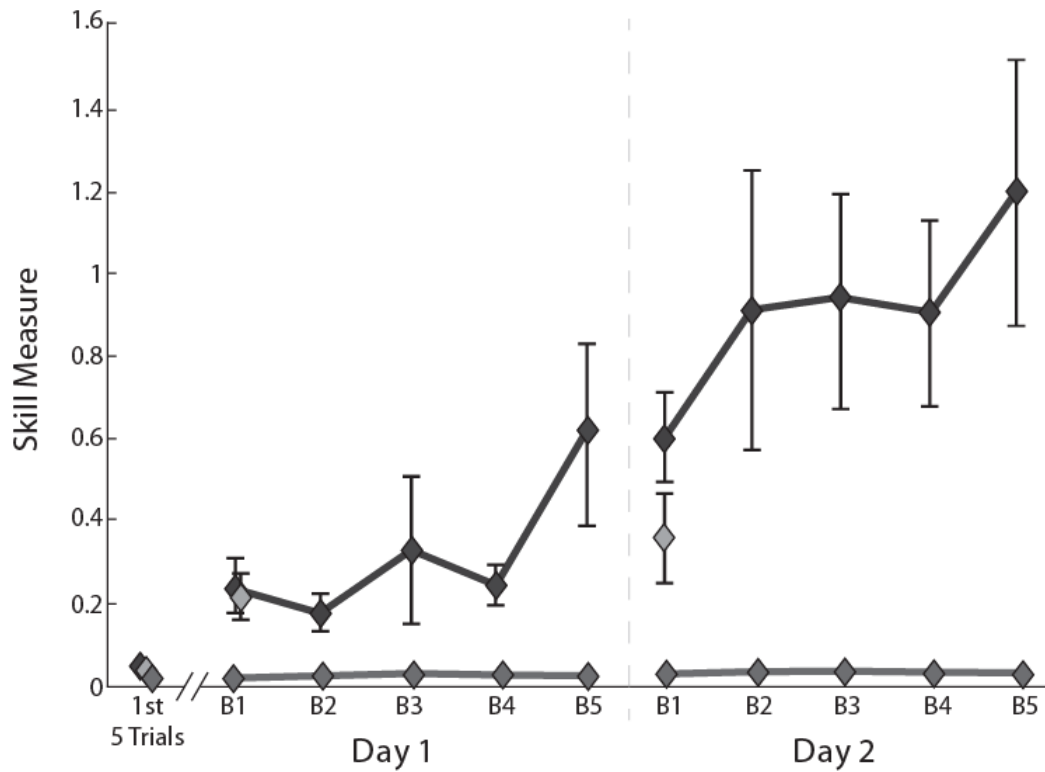


Figure 3.2: Skill learning. Skill performances are presented for the Long (blue), Short (green), and Random (red) groups. The vertical grey solid line represents the separation between Day1 and Day2 of training. The y-axis shows the skill measure and x-axis depicts the blocks of training. We also present the mean \pm SEM of the first 5 trials for all groups. Note that all individuals started with similar skill level, but with more training the Long and short groups continue to improve their skill measure. Random group participants do not improve their skill measure across training sessions. Data are means \pm SEM.

The short and long training groups learned the skill, but not the random group.

To assess skill learning, we compared skill measure (see equation 1 in Methods) differences across training blocks and days in the Long, Short and Random training groups. Given the different number of observations between the 3 groups, first we compared the performance of block 1 across the 3 groups for day 1 only. Performance was significantly better in the Long ($p = 0.023$) and the Short groups ($p = 0.020$; Figure 3.2) relative to the Random Group. Moreover, there was no early performance difference between the Short and Long groups ($p > 0.5$), suggesting that individuals from these two groups were similar at acquiring the skill. Additionally, the skill measure for all three groups was similar after the first five trials ($p > 0.5$). This indicates that all groups started with a similar level of knowledge of the task and that learning occurs within block 1 for the Long and Short groups only. We also compared the skill measure of each block across both training sessions between the Long and Random groups, where amount of performance was matched. ANOVA_{RM} revealed a significant difference for GROUP ($F_{(1,16)} = 6.859$; $p=0.019$), DAY ($F_{(1,16)} = 4.878$; $p = 0.042$) and DAY x GROUP interaction ($F_{(1,16)} = 4.779$; $p=0.044$). These results show that participants exposed to a consistent sensorimotor mapping acquired the skill, whereas individuals who trained on an inconsistent mapping were unable to improve their performance.

CBI changes are specific to early skill learning

We compared the amount of CBI changes across the groups before, during and after the skill training blocks. Since stimulation time points before training (Pre) and after 1 block of training (P1) was matched between each group, we first assessed the early changes in CBI across block 1 for each day. ANOVA_{RM} revealed a significant effect of CBI for TIME ($F_{(2,26)} = 21.106$; $p<0.001$) and TIME x GROUP interaction ($F_{(2,26)} = 5.684$; $p=0.009$). CBI following early skill learning in the Long and Short groups were significantly decreased compared to Pre ($p<0.001$,

$p=0.001$, respectively; Figure 3.3), whereas we found no difference for the Random group ($p = 0.955$). This finding shows that CBI changed early on, only when subjects learn.

To determine whether CBI returns towards baseline values late in skill learning, we additionally compared CBI between Long and Random groups across days for each time point (Pre, P1,..., P3). ANOVA_{RM} revealed a significant effect of CBI for TIME ($F_{(3,48)} = 3.362$; $p=0.026$), GROUP ($F_{(116)} = 7.462$; $p=0.015$) and TIME x GROUP interaction ($F_{(3,48)} = 3.597$; $p=0.02$). Subjects in the Long training group showed significant CBI changes from pre-training to early skill learning (Pre-P1, $p=0.001$; Pre-P2, $p=0.003$), but no differences from pre-training to late skill learning (Pre-P3, $p=0.307$). This result suggests that the reduction of CBI occurs early during learning with a progressive return towards baseline despite further overall skill improvements. Interestingly, we found no significant differences in DAY x TIME ($F_{(3,48)} = 0.086$; $p=0.967$) or DAY x TIME x GROUP ($F_{(3,48)} = 0.163$; $p=0.921$) in the Long and Random groups, indicating the CBI dynamic changes during learning were similar across training session.

Importantly, the changes in CBI were not due to simple changes in test stimulus responses from M1. MEP amplitudes generated by the unconditioned TS were not different over TIME or SESSION in all groups (Table 1; all $p > 0.1$). This is because we controlled for potential M1 excitability changes by adjusting the stimulator intensity. Thus, our results indicate that changes in CBI, probed by CB-M1 stimulation, are present early on within a skill training session, but not late.

CBI changes are proportional to skill acquisition

Previous investigations have shown AtDCS over the cerebellum increased on-line skill learning, with the most noticeable difference after the first training block (Cantarero et al., 2015).

Moreover, changes in CBI were previously found to correlate with the amount of locomotor

adaptation (Jayaram et al., 2011). Here, we tested whether a similar relationship between change in CBI and early skill learning exists. To calculate early skill improvement, we divided the first training block in half and re-calculated two new skill measure scores for each half. Thus, we subtracted the skill score from the second half vs. the first half (within block 1 skill improvement) and performed a correlation analysis with CBI changes between pre-training and after the first training block (Pre-P1) in the Long and Short training groups. We found a relationship between skill improvement within the first block and early change in CBI ($r=0.518$; $p=0.001$; Figure 3.4), where participants who showed more CBI changes were better at acquiring the skill.

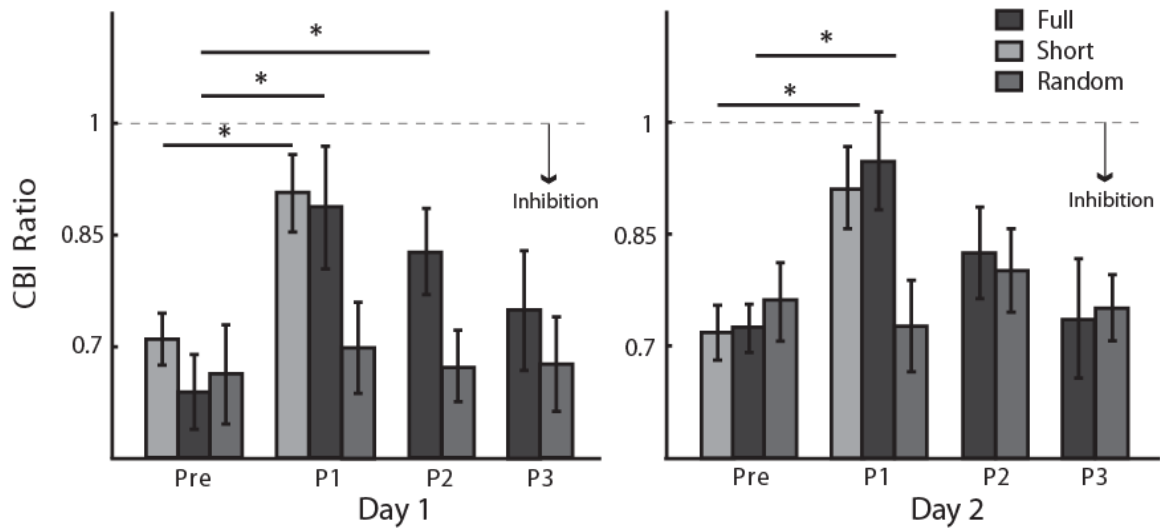


Figure 3.3: Cerebellar Excitability (CBI). Bar graphs show the mean CBI amplitude for Long (blue), Short (green), and Random (red) groups. The y-axis represents the CBI Ratio and the x-axis represents stimulation time-points before (Pre), during (P1, P2) and after (P3) training on each day. The ratio increases (less inhibition) early in learning for both the Long and Short groups, but not for the Random group. Data are means \pm SEM. $*p \leq 0.05$

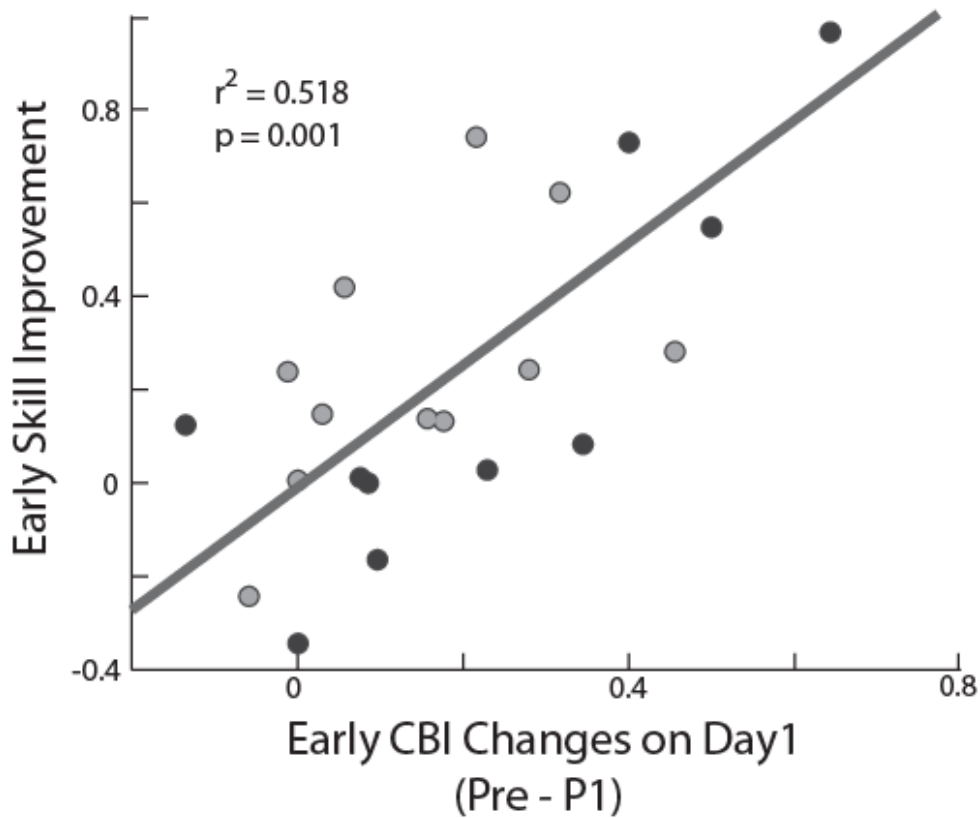


Figure 3.4: Correlation between early CBI changes and behavior. y-Axis represents early skill acquisition on day 1, and the x-axis represents early CBI change (P1-Pre). Blue circles represent individual subjects of the Long group and the green circles correspond to Short group individuals. To calculate early skill acquisition, we split the first behavioral block (B1) in half and computed two separate skill measures (first 15 trials vs. next 15 trials) and calculated the difference in skill measure. Note that both groups experienced the same amount of trials to allow for this comparison. Subjects who had the largest CBI change improved the most in the first block of D1.

Occlusion of LTP-like plasticity occurs later during the skill practice

We compared the amount of AtDCS-induced potentiation before and after training in the Long, Short and Random groups. We found a significant effect of AtDCS on MEP amplitudes for TIME ($F_{(1,52)} = 32.892, p < 0.01$), TIME x GROUP ($F_{(1,52)} = 16.618, p < 0.01$) and DAY x TIME x GROUP interactions ($F_{(4,52)} = 2.629, p = 0.045$). All groups experienced an increase in MEP amplitudes in the baseline session ($p < 0.01$). However, while the Short and Random groups showed similar increase in MEP amplitudes following application of AtDCS after the training ($p < 0.01$), the Long group failed to show differences in MEP amplitudes ($p = 0.186$, Figure 3.5a). To further quantify the amount occlusion of LTP-like capacity after training, we computed an occlusion index (OI) (see equation 2 in Methods) for each group. The OI represents the difference between the peak MEP response following application of AtDCS at baseline and following AtDCS after training. We found that only the Long training group experienced occlusion (i.e. a large OI) in both days ($F_{(1,18)} = 14.634, p = 0.440$), but not the short and random training groups ($F_{(1,20)} = 0.621, p = 0.001$; $F_{(1,14)} = 0.354, p = 0.561$). Altogether, these results indicate that more skill training leads to interference or occlusion of AtDCS potentiation effects.

Motor skill retention is proportional with the amount of occlusion

We conducted a correlation analysis between offline behavioral changes in the Long training group (Day2 B1-Day1 B5) and the first day OI. We found that those who retained the most experienced the largest occlusion ($r=0.464$; $p=0.025$; Figure 3.6). This finding is consistent with prior studies showing that occlusion is proportional to skill retention (Cantarero et al., 2013a&b).

M1 excitability changes are not specific to learning

First, we compared early s1mV changes across days for block 1 in all groups. ANOVA_{RM} showed a significant effect of s1mv changes for TIME ($F_{(2,26)} = 10.256$; $p=0.004$), but no differences for

GROUP ($F_{(2,26)} = 0.169$; $p=0.845$), DAY ($F_{(1,26)} = 0.906$; $p=0.350$) or their TIME x GROUP interaction ($F_{(2,26)} = 0.793$; $p=0.463$).

Second, we used ANOVA_{RM} to compare s1mV changes in the later time points in the Long and Random groups. Again, we found a significant difference for TIME ($F_{(3,48)} = 6.022$; $p=0.001$), but no differences for GROUP ($F_{(1,16)} = 0.229$; $p=0.639$), DAY ($F_{(1,16)} = 0.122$; $p=0.732$) or their TIME x GROUP interaction ($F_{(2,26)} = 0.511$; $p=0.677$). These results indicate that changes in s1mV excitability were not specific to learning, but rather due to motor execution.

Finally, we performed a similar analysis for SICI. When comparing early SICI changes for all groups, ANOVA_{RM} did reveal significant effect of TIME ($F_{(1,26)} = 5.546$; $p=0.027$), but there were no differences within GROUP ($F_{(2,26)} = 0.421$; $p=0.661$) or between TIME x GROUP interaction ($F_{(2,26)} = 0.508$; $p=0.607$). When comparing SICI in the other time points in the Long and Random training groups we found no significant changes (all $p>0.2$). Thus, our results showed that early changes in SICI are due to motor execution or the simple passage of time, but not to skill learning.

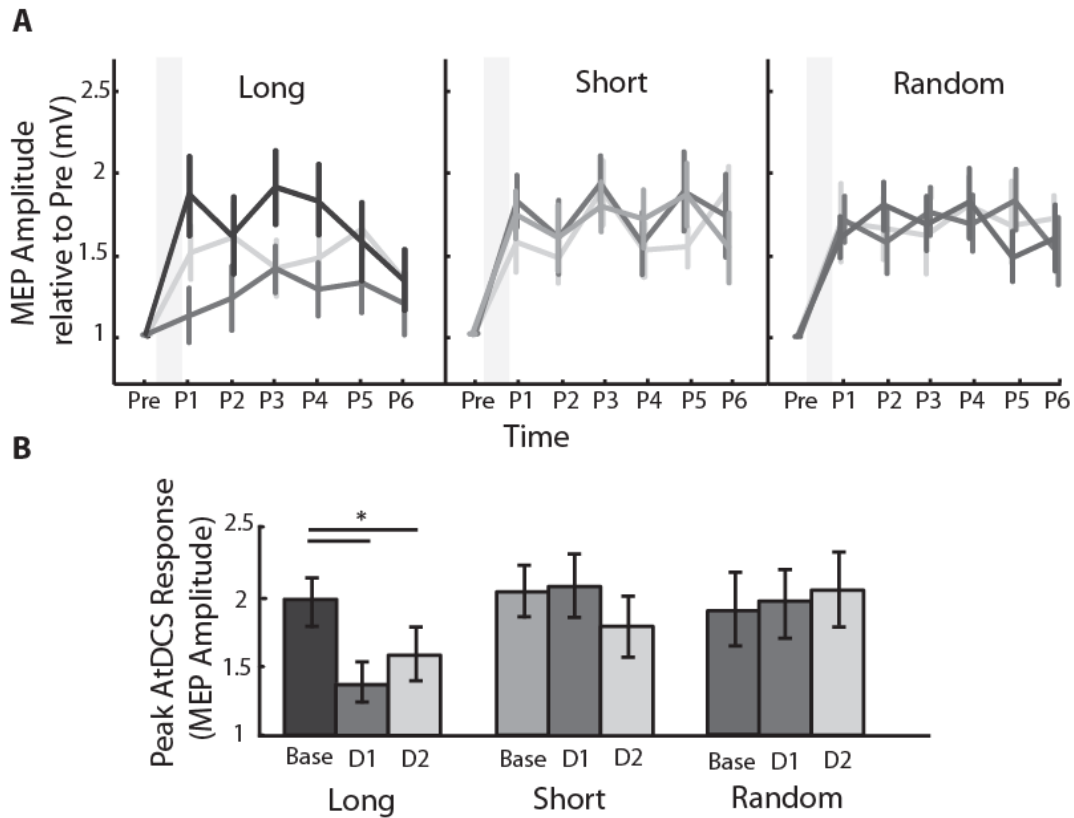


Figure 3.5: M1 LTP-Like Plasticity Aftereffects. (A) MEP amplitude ratios for pre and post AtDCS. y-Axis represents the mean MEP amplitude normalized to the pre-AtDCS MEP amplitude, and the x-axis presents TMS measurements taken before application of AtDCS (pre), immediately after AtDCS (Post 1, P1) and repeated every 5 minutes up to 25 min after AtDCS (P2...P6). The left, middle and right portion of the graph depicts MEP amplitudes for individuals of the Long, Short and Random group respectively. Colored lines represent the timeline of MEP amplitude responses for all subjects on Day0 (baseline session), whereas dark grey and grey present responses after Day1 and Day2 training session. The gray shadowed columns represent the time when AtDCS was applied. Note, all groups demonstrated an increase in excitability in response to AtDCS for the baseline session. (B) The bar graphs show the peak MEP amplitude response following AtDCS for each session. Only participants of the Long group showed significant occlusion of LTP-like plasticity after each training session when compared to baseline responses. Data are means \pm SEM. * $p \leq 0.05$.

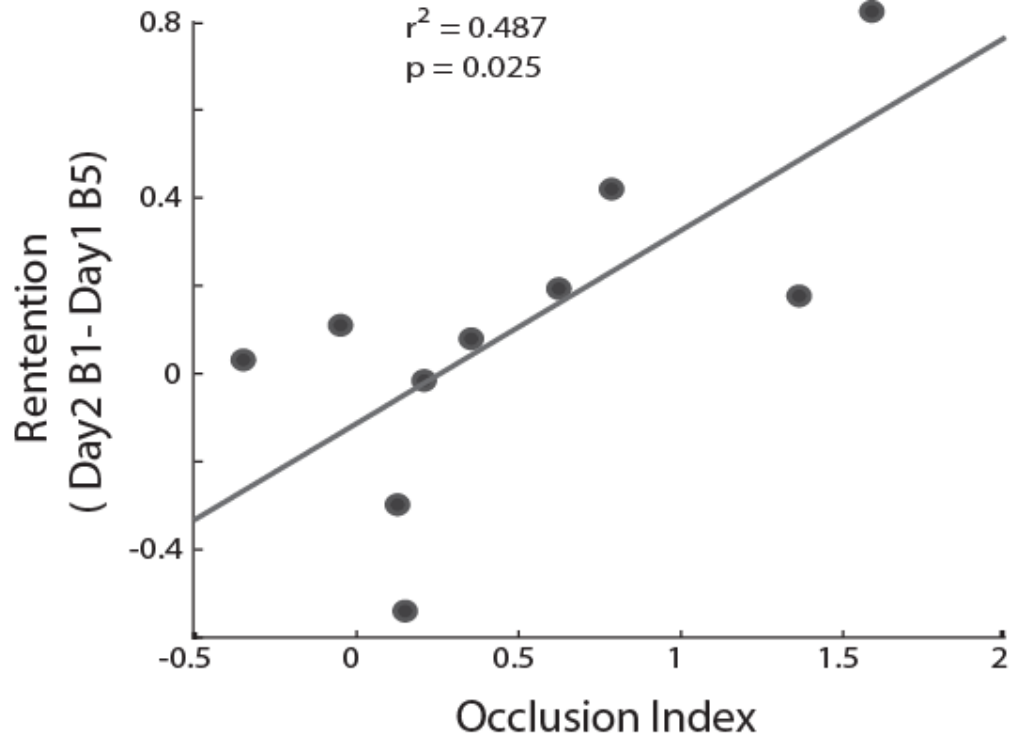


Figure 3.6: Correlation between Occlusion of LTP-like Plasticity and Behavior. Y-axis represents the retention of the skill measure and x-axis represents the Occlusion Index. Blue circles represent individuals of the Long group. Note that subjects who had the largest OI after training had the best skill retention measured on day 2.

3.3 Discussion

Our study demonstrates an important temporal dissociation in the neurophysiological role the cerebellum and M1 play during skill learning. Specifically, we found a reduction in CBI early in skill learning, but not late. The magnitude of the CBI change correlated with early skill acquisition; participants who initially improved the most on their ability to perform a new skill had the largest reduction of CBI. On the other hand, occlusion of M1 LTP-like plasticity only occurred after a significant amount of training has taken place. In addition, we found that those participants who occluded the most on their first training session had better skill retention on the following day. Critically, these changes were only observed with learning, but not when subjects performed a randomized task that does not lead to learning. Indeed, overall motor execution, rather than learning, resulted in primary motor cortex excitability changes and a reduction in SICI.

There is growing evidence from both animal and human studies demonstrating that learning induces changes in synaptic plasticity in both the cerebellum (Anderson et al., 1996; Kleim et al., 1997; Torriero et al., 2011; Jayaram et al., 2012; Schlerf et al., 2012; Medina and Lisberger, 2008; Yang and Lisberger, 2014) and the primary motor cortex (Pascual-Leone et al., 1995; Classen et al., 1998; Kleim et al., 1998; Ziemann et al., 2004; Rosenkranz et al., 2007; Lepage et al., 2012; Cantarero et al., 2013a&b; Avanzino et al., 2015).

Specifically, the cerebellum is critical to error-based learning tasks, such as motor adaptation, where motor commands are rapidly adjusted for new predictable demands (Dochin et al., 2012; Wolpert and Kawato, 1998; Diedrichsen et al., 2010). On the other hand, M1 has an important role in motor retention (Muellbacher et al., 2001; Reis et al., 2009; Baraduc et al., 2004; Hadipour-Niktarash et al., 2007). In this region, Hebbian, such as use-dependent learning, and operant reinforcement mechanisms where learning is influenced by the repetition of prior successful

movements has been described (Ziemann et al., 2004; Stefan et al., 2006; Rosenkranz et al., 2007). Despite the distinct roles for the cerebellum and M1 during skill learning, physiological mechanisms related to these structures have never been tested directly in the same task throughout the different stages of skill learning.

As we have previously shown in motor adaptation studies (Jayaram et al., 2011; Schlerf et al., 2012, 2014), we found that CB-M1 connectivity changed early in the (long and short) skill-learning groups only, and returned to baseline levels as training proceeded. Given that we controlled for M1 excitability modifications when assessing CBI (by adjusting TMS intensities) and the fact that M1 excitability changed similarly in all groups and in all the post-training time points, we interpreted the specific CBI findings as driven by cerebellar plasticity. This suggests that error-based learning processes, which heavily contribute to motor adaptation tasks, might play a similar role early on during skill acquisition. We reason that to be able to learn a new skill first it is crucial to calibrate the appropriate motor outputs to interact with either the device being used and or the dynamics of the environment. In our experimental setup, learning the relationship between the force transducer and cursor movement could be interpreted as similar to a perturbation of a previously known map in a motor adaptation task. Participants have to account for this force-visual display map and adjust their movements to make a successful action. We therefore conclude that learning this mapping (i.e. the dynamics of the task) is what drives early engagement of the cerebellum, as denoted here by CBI changes. Indeed, we found that when participants performed the skill on an inconsistent trial-to-trial force-distance map task, CBI did not change. This finding is consistent with previous studies that found AtDCS over the cerebellum reduced the error rate when learning the same task lead to improved skill acquisition¹⁰, as well as visuomotor, locomotor and force field adaptation (Galea et al., 2011; Jayaram et al., 2012; Herzfeld et al., 2014). We also predicted that the early CBI changes should correlate with the amount of early skill acquisition. Of note, although we did not find an

association between CBI and error-rate, early CBI changes correlated with improvement in early skill measure scores, which reflects improved trade-off between error-rate and movement time.

Previous animal and human investigations have shown that after learning a motor task the ability to artificially induced LTP-like changes is reduced, an effect explained by the saturation of the synaptic modification range (Rioul-Pedotti et al., 1998, 2000, 2007; Ziemann et al., 2004; Rosenkranz et al., 2007; Lepage et al., 2012; Avanzino et al., 2015). According to this model, if skill learning uses up some of the plasticity available at motor cortical synapses, then additional synaptic strengthening through LTP-like mechanisms should be reduced. In other words, there is a reduced capacity for further LTP-plasticity after learning. We observed this phenomenon, named occlusion of LTP-like plasticity, only in participants who were able to learn the motor task. This result supports the idea that motor learning-induced plasticity interacts with NIBS protocols that lead to plastic changes in M1 (Ziemann et al., 2004; Stefan et al., 2006; Rosenkranz et al., 2007; Cantarero et al., 2013a&b). Consistent with Cantarero and others (2013a), we also found an association between the magnitude of occlusion on day 1 and retention of the skill on the next day, supporting the evidence that occlusion is a critical mechanism of skill retention. Of note, previous studies have shown that retention of the SVIPT motor task is unaffected by AtDCS application immediately after training (Cantarero et al., 2013a&b). In addition, while recent studies have found that consecutive AtDCS sessions can lead to cumulative increases in cortical excitability (Alonzo et al., 2012; Galvez et al., 2013), we found no differences in the baseline TMS measures across days. This indicates that any potential tDCS aftereffects on excitability were not present on Day 2. Furthermore, although the intra-subject variability of AtDCS aftereffects remains poorly understood, it might be a potential limitation of the occlusion measurement. However, the lack of AtDCS potentiation was present only in the Long training group, but not in the Short and Random groups. Thus, despite this potential limitation, the effect size found in the Long group was larger and beyond the variability that can be expected in all

groups. This supports the concept that occlusion of LTP-like plasticity is only observed during learning, but not with simple motor execution.

In our study, however, we found that occlusion of LTP-like mechanisms were not present early on during skill learning. A possible explanation for this result is that early skill learning may rely more on error-dependent forms of learning. This would suggest that at early stages of skill learning error-based learning is weighted more to acquire the dynamics of the task before movements are worth encoding. This is not to say that other mechanisms within M1 that support early rapid plasticity, such as unmasking of pre-existing connections (Jacobs and Donoghue, 1991) or awakening of silent synapses by insertion of postsynaptic AMPA receptors (Malinow et al., 2000) are not occurring in the early stages of learning. Alternatively, it is also possible that some synaptic strengthening via LTP-like mechanisms is taking place early on during learning, but our measures to detect these changes are not sensitive enough.

In this study, we show that occlusion also occurs after significant performance improvement on the second training session. This result is in agreement with previous studies in rats, which showed occlusion of LTP plasticity up to five days after skill learning (Rioult-Pedotti, 2007). In humans, however, Rosenkranz and others (2007) failed to observe persistent LTP/LTD-like plasticity after five days of training. The authors suggested that this effect could be attributed to learning via a different mechanism other than LTP/LTD-like plasticity; however, it is also possible that in that study little learning took place in the last session of training. This would be consistent with our random skill-training group, which did not experience learning or occlusion.

Previous studies have shown that M1 excitability and short-interval intracortical inhibition (SICI) changes with motor learning (Perez et al., 2004; Coxon et al., 2014). However, these investigations did not control for movement execution vs. true performance improvement. Here, we found that both the learning and non-learning groups experienced changes in corticomotor

excitability and SICI. This indicates that motor execution leads to M1 excitability changes, an effect that is not necessarily tied to learning. This observation is consistent with our previous findings in healthy individuals learning motor adaptation tasks (Jayaram et al., 2011; Schlerf et al., 2012). Of note, it is possible that other forms of SICI (e.g. SICI at 1msec) not tested in this study might also modulate with learning (Coxon et al., 2014), a question that needs to be addressed in future studies. Altogether, these results indicate that occlusion of M1-AtDCS effects may be a better marker for learning-related plasticity rather than simple changes in M1 excitability or SICI.

This study demonstrates the temporal dynamic role of the cerebellum and M1 when learning new motor skills. Our results suggest that skill learning, which likely relies on many different forms of learning, incorporates early on cerebellar dependent error-based processes, and later on engages M1-LTP like plasticity that might be linked to other forms of learning such as reinforcement or use-dependent. This is in agreement with recent work that suggests that learning different motor tasks involves error-based and other forms of learning (Huang et al., 2011; Haith and Krakauer, 2013). Altogether, our findings indicate that early on during motor skill learning, cerebellar dependent-learning mechanisms (i.e. error-based process) are needed to learn the task dynamics before the primary motor cortex, incorporating other forms of learning (i.e. reward-based or use-dependent), is engaged. This concept is critical to design rational interventions that target specific neural regions to affect specific processes to augment motor learning.

3.3 Methods

We recruited a total of 29 young, healthy right-handed individuals (mean age = 22.04 ± 0.56 years; 16 female) with no history of neurological disorders. Participants were screened prior to enrollment in the study to ensure that they did not have conditions that would exclude them from non-invasive brain stimulation. Exclusion criteria included the use of nicotine, alcohol,

recreational drug use and absence of prescribed medication affecting the central nervous system, all of which may alter plasticity and motor learning. All participants provided written informed consent to participate in this study and the Johns Hopkins School of Medicine Institutional Review Board (IRB) approved all experimental procedures. All experiments were performed in accordance with relevant guidelines and regulations.

Behavioral Measurements

Motor Skill task: Sequential Visual Isometric Pinch Task (SVIPT)

As previously described (Reis et al., 2009; Cantarero et al., 2013a&b), participants were seated in front of a computer screen and held a force transducer between the right thumb and index finger. Pinching the force transducer controlled the movement of an on-screen cursor with an overall goal to move the cursor between a HOME position and 5 targets (the sequence of movements: HOME-1-HOME-2-HOME-3-HOME-4-HOME-5). This sequence was held consistent throughout training. Long and Short training groups were exposed to a consistent and learnable logarithmic sensorimotor mapping between forces applied to the transducer and cursor movement, whereas Random group individuals were exposed to a variation of the SVIPT where the force-distance mapping and sensitivity was randomized trial-by-trial. All participants were informed that both movement time and accuracy contributed to the overall skill score and were encouraged to improve in both domains. Movement time was considered as the total time from movement onset until target 5 was reached. A trial was considered correct only if participants hit each target in the correct order. Thus, accuracy for a trial was calculated in a binary fashion (i.e. no error or error), regardless of participants committing multiple errors.

To assess the skill performance of participants, we used a speed-accuracy trade-off function (SAF). The function used to estimate the SAF throughout performance is the skill measure described in equation 1:

$$1 : Skill Measure = \frac{1 - \text{error rate}}{\text{error rate}(\ln(\text{movement time})^b)}$$

As done in previous studies (Reis et al., 2009; Cantarero et al., 2013a&b; Wymbs et al., 2016), average movement time and error rate (proportion of trials with at least 1 error) were calculated for each block of 30 trials, and the value of b was held constant at 5.424.

EMG Recording

Electromyographic (EMG) activity was captured using electrodes placed over the right first dorsal interosseous muscle (FDI) muscle. EMG signals were sampled at 2 kHz, amplified at 1 kHz and band-pass filtered (10–500 Hz) using an amplifier (Octopus AMT 8; Bortec Biomedical, Alberta, Canada) and data acquisition software (Signal 4.02; CED, Cambridge, England). Data was stored on another computer to complete off-line analysis using a variety of custom Matlab scripts (MathWorks, MA, USA).

Transcranial magnetic stimulation (TMS)

For all TMS measures, we used a 70 mm-diameter figure-of-eight TMS coil (Magstim 200²) over M1. We used a neuronavigation system (BrainSight; Rogue Research) to ensure stimulation over the desired M1 location occurred at the same spot from session to session. To do this, we identified and marked the spot over the M1 with the best representation of the right FDI muscle. In this location, we found the resting motor-threshold (rMT) for the FDI, or the minimum intensity needed to elicit an MEP of 50 uV on 5 out of 10 pulses (Rossini et al., 2015). The rMT values collected from individuals within each group can be found in Supplementary Table S1

online. For all analysis, we recorded peak-to-peak amplitudes of motor evoked potentials (MEP) using electromyography (EMG).

Transcranial Direct Current Stimulation (tDCS)

Using a Chattanooga Ionto Phoresor II Auto device (model PM850; IOMED, UT, USA), we delivered anodal transcranial direct current stimulation (AtDCS) through two sponge 25 cm² electrodes soaked in a saline solution. Electrodes were placed over the contralateral (left) M1 corticomotor representation of the right FDI muscle and the ipsilateral supra-orbital area. Stimulation was applied for 7 minutes at an intensity of 1mA as participants were instructed to relax and remain seated. We used AtDCS as our LTP-like inducing protocol since this form of stimulation can increase cortical excitability via NMDA receptor, BDNF and calcium-dependent mechanisms in humans (Liebetanz et al., 2002; Nitsche et al., 2003; Fritsch et al., 2010), and is capable of assessing occlusion after motor learning.

Recent studies have indicated that AtDCS aftereffects can show variability between-subjects (Lopez-Alonso et al., 2014; Dyke et al., 2016; Horvarth et al., 2016), where some individuals do not show potentiation of MEPs after AtDCS application. We therefore screened out non-responders to AtDCS based on post-AtDCS MEP changes in the baseline session. A non-responder was classified when the mean of normalized MEP amplitude across each post-AtDCS time points did not exceed 1.1 (i.e., smaller or equal than pre-AtDCS MEP). Six individuals did not meet this criterion and were therefore excluded from the study. Importantly, the intra-subject consistency of AtDCS responses across multiple sessions is poorly understood, but might be a factor that can influence the group occlusion magnitude.

Measures of Cerebellar Excitability (CBI)

For cerebellar stimulation, we placed a double-cone coil (110mm mean diameter) 3cm lateral to the inion, with the stimulator current directed downward (Ugawa et al., 1995). As previously

described, we assessed CBI by using a paired-pulse stimulation paradigm (for review, see Celnik 2015). For each measurement, we collected 20 TMS test stimuli (TS) over left M1. In half of these exposures, selected randomly, a TMS conditioned stimulus (CS) was delivered over the right cerebellum 5 ms prior to the TS. Accordingly, a total of 10 CS+TS and 10 TS pulses were administered. CBI was calculated as the ratio of the mean MEP amplitude in the CS+TS relative to TS. The CS maximum stimulator output (MSO) for all participants was set to 70% since no MEPs were evoked via brainstem pathways at 75% MSO. Throughout each stimulation time point, the intensity of the TS was adjusted to evoke an MEP of ~ 1Mv. The mean stimulus intensity for the adjusted TS throughout this study was less than 1% (see Supplementary Table S2 online). Thus, the CBI response reflects cerebellar inhibition of M1 regardless of changes in excitability of M1 after training.

Measures of Primary Motor Cortex (M1) Excitability

To assess corticomotor excitability, we determined the MSO intensity needed to evoke an MEP amplitude of ~ 1mV at rest (s1mV). At this MSO intensity, we recorded 10 MEPs at each stimulation time point. Furthermore, to assess short-intracortical inhibition (SICI), we used an interstimulus interval of 2 ms between subthreshold CS and suprathreshold test stimulus for the paired pulse paradigm (Kujirai et al., 1993). We set our subthreshold CS set at 80% of rMT and suprathreshold TS intensity set to elicit ~ 1mV (Ziemann et al., 1996; Heise et al., 2014). Similar to CBI, we established SICI as a ratio of 10 CS+TS over 10 TS MEPs. The intensity of the TS used to measure SICI was adjusted to the same intensity used for CBI.

Measures of LTP-like saturation (Index of Occlusion)

To assess occlusion after motor performance, we applied AtDCS as done by Cantarero and others (2013a&b). On each experimental day, prior to applying AtDCS, we collected 10 MEPs at the intensity used for s1MV. If participants M1 excitability increased after training, we adjusted the

s1mV intensity to elicit ~1mV amplitude and used this adjusted intensity for all post AtDCS measurements. This allowed us to compare potentiation effects of AtDCS at rest and after training. After applying stimulation for 7 minutes, we recorded 10 MEPs (at s1mV or adjusted s1mV intensities) every 5 minutes until 30 minutes post AtDCS application. At each time point, we averaged the recorded 10 MEP amplitudes and normalized these values to the average of 10 MEP amplitudes prior to AtDCS application.

To quantify the magnitude of occlusion, we calculated the occlusion index (OI) for each participant. We selected the peak MEP amplitude after AtDCS application normalized to the MEP amplitude prior to AtDCS application for each subject. The peak MEP amplitude was selected as the largest mean MEP on any one of the six post measurement time points. This measurement was done after each session: Baseline (no training), Day1 and Day2. The OI represents a comparison between the rest and post training measures, thus we subtracted baseline day amplitudes to both training session amplitudes in equation 2:

$$2 : OI_{1,2} = \text{Baseline} \left[\frac{\text{Post Peak MEP}}{\text{Pre MEP}} \right] - \text{Day}_{1,2} \left[\frac{\text{Post Peak MEP}}{\text{Pre MEP}} \right]$$

This measurement was used as an index of how much potentiation plasticity was used during training, where larger values for the OI are indicative of more occlusion, which would imply more resources were used to induce plasticity changes during learning.

Data Analysis

For all data statistical analyses, SPSS (IBM; Version 20) was used and effects were considered significant if $p \leq 0.05$. All data are given as means \pm SEM. We used separate polynomial nested repeated measures of ANOVA (ANOVA_{RM}) for all behavioral and physiological measures.

Furthermore, we used Mauchly's sphericity test to validate that the variances between each group

tested in ANOVA_{RM} are equal. When appropriate, Bonferroni corrected *post hoc* analysis was done to account for multiple comparisons.

We used skill measure as our primary behavioral outcome measure. To assess differences in early performance on Day 1, we used a one-way ANOVA_{RM} with between factor GROUP (Long, Short, Random) and within factor TIME (B1). Skill measure scores were also compared across participants who engaged in extended training (i.e. Long and Random). Here, we used ANOVA_{RM} with GROUP (Long, Random) as the between factor and DAY (Day1, Day2) and TIME (Block1, Block2...Block5) as within-factors.

To determine early changes in CBI, SICI, and s1MV, we used ANOVA_{RM} with GROUP (Long, Short, Random) as the between factor and DAY (Day1, Day2) and TIME (PRE, P1) as within factors. In a separate analysis, we assessed these TMS measurements when all stimulation time points were matched between GROUP (Long and Random) across DAY (Day1, Day2) and TIME (PRE, P1, P2, P3).

Using the peak-to-peak MEP amplitudes as the primary outcome measure, the amount of potentiation plasticity aftereffects via AtDCS application was compared using ANOVA_{RM} with the between factor GROUP (Long, Short, Random), and the within factors DAY (Base, Day1, Day2) and TIME (Pre-AtDCS, mean of Post-AtDCS [P1, P2, P3, P4, P5, P6]). Additionally, we used peak AtDCS response as our primary measure to assess how much LTP-like plasticity was used during training. For each day, peak MEP amplitudes aftereffects were compared using ANOVA_{RM} between each GROUP (Long, Short, Random) and within each DAY (Base, Day1, Day2).

To determine associations between early cerebellar physiological changes and behavior (Day1), we combined data from both Long and Short groups and performed a correlation analysis between early changes in CBI (P1-Pre) and early skill improvement within the first block (first 15

trials vs. next 15 trials). Similarly, to determine associations between OI_1 and behavior (offline changes Day2 B1- Day1 B5) in the Long group, we also performed correlations using Spearman's ρ .

Chapter 4 – The components of motor skills are learned via different neurophysiological mechanisms

4.1 Introduction

Successfully executing a motor skill, such as hitting a baseball, requires our brain to develop an understanding of how to interact with a new object or environment (e.g. weight of the bat, field conditions), as well as to coordinate an appropriate sequence of movements (i.e. fluid swing). Despite the enormous amount of computations needed to acquire each of these motor components, we are seamlessly capable of learning them simultaneously. We posit that skill learning is accomplished by engaging different brain regions, each acquiring distinct motor skill components and relying on different physiological mechanisms.

Performing smooth and accurate movements is thought to rely on internal forward models--representations of the body capable of predicting sensory consequences of our own actions (Wolpert et al., 1998; Desmurget and Grafton, 2000; Todorov, 2004; Shadmehr and Krakauer, 2008). Electrophysiological (Palasar et al., 2006; Herzfeld et al., 2015), imaging (Diedrichsen et al., 2005; Schlerf et al., 2012), and patient studies (Martin et al., 1996; Lang and Bastian, 1999; Maschke et al., 2004; Smith and Shadmehr, 2005; Xu-Wilson et al., 2009; Bhanpuri et al., 2013) have all implicated that the cerebellum acquires and maintains internal models. Human motor adaptation studies have indicated that reducing sensory-prediction errors (error-based learning) leads to the formation of internal models in the cerebellum (Tseng et al., 2007; Taylor et al., 2010; Izawa et al., 2012). Similarly, animal and human research have shown this type of learning leads to neurophysiological changes within the cerebellum (Medina and Lisberger, 2008; Yang and Lisberger, 2013; Jayaram et al., 2011; Schlerf et al., 2012, 2014). Although, prior work has evaluated error-based learning by introducing systematic perturbations, this learning mechanism likely contributes to forming an internal model representation of a novel task (Bastian, 2008;

Haith and Krakauer, 2013; Taylor and Ivry, 2014). Here, we investigated whether acquiring de novo a sensorimotor map (or internal model) that is fundamental to successfully perform a skill task also engages the cerebellum.

Linking multiple elements into a single action and optimizing performance of a sequence of movement has been suggested to rely on the cerebellum and motor cortical regions (Hikosaka et al., 2002; Doyon et al., 2003; Doyon et al., 2009; Steele and Penhune, 2012). For instance, cerebellar patients' show learning impairments in novel coordination patterns and sequences (Pascual-Leone et al., 1995; Doyon et al., 1997; Molinari et al., 1997; Gomez-Beldarrian et al., 1998; Shin and Ivry 2003; Diedrichsen et al., 2005), and damaging cerebellar nuclei in monkeys impairs automatization of motor sequences (Passingham and Nixon, 2001). Although these studies indicate cerebellar contributions to acquiring sequences the neurophysiological changes associated to this remain largely unknown. On the other hand, the primary motor cortex (M1) is also known to play an active role in acquiring and encoding movement sequences (Lu and Ashe, 2005; Matsuzaka et al., 2007; Wiester and Diedrichsen, 2013). Repetition of the same movement pattern rapidly alters the output organization of M1 (Pascual-Leone et al., 1995; Nudo et al., 1996; Classen et al., 1998; Kleim et al., 1998; Liepert et al., 1999), a process thought to rely on mechanisms of synaptic efficacy, such as long-term potentiation (LTP) (Rioul-Pedotti et al., 1998, 2000; Harms et al., 2008). Interestingly, LTP-like plasticity of M1 has been described as a neurophysiological phenomenon associated with motor skill learning and retention in humans (Reis et al., 2013; Cantarero et al., 2013a&b; Spampinato and Celnik, 2017). These studies, however, assessed learning of complex skills including both sensorimotor maps and sequences. In other words, prior investigations cannot disentangle the role of M1 and or the CB when learning different skill components.

Since learning motor skills involves acquiring the sensorimotor map and sequence components simultaneously, here we deconstructed a skill task to assess the distinct physiological

contributions of the cerebellum and M1 when participants learn the skill components separately. We predicted that learning a sensorimotor map results in modulation of cerebellar excitability, but not in M1 LTP-like plasticity changes; whereas learning a sequence of movements leads to cerebellar excitability changes and M1 LTP-like plasticity. We argue that the nature of motor components that constitute a skill, determines which brain regions and physiological mechanisms mediate the overall motor skill learning. This raises the question whether developing interventions targeting multiple brain regions, rather than a single-site, results in a more efficient modulation of motor learning.

4.2 Results

In experiment 1 we investigated whether changes in cerebellar excitability and M1 LTP-like plasticity occurred as participants learned a new sensorimotor map that is a critical component of a skill task. Here, participants move a computer-cursor to an individual target by producing an isometric pinch force contractions. The relationship between the cursor displacement and force (sensorimotor map) was logarithmic (Figure 4.1a). We designed the task to expose participants to the entire logarithmic map by changing the target location on each trial to a random position along the task (monitor) space. Participants were instructed to hit the target as fast and accurately as possible. They only had one attempt per trial for each target location (i.e. subjects were not allowed to make force adjustment). Following the sensorimotor map training, participants were then asked to complete a sequential visuomotor isometric pinch task (SVIPT) where cursor movements are navigated using the same sensorimotor map through a sequence of five targets presented on the screen (Figure 4.1b). Recent studies have suggested that early learning of this sequential skill task relies on cerebellar-dependent error-based learning mechanisms, before other forms of learning, such as repetition and reinforcement are engaged (Cantarero et al. 2015; Spampinato and Celnik 2017). Thus, we predicted that having participants learn the sensorimotor

map prior to incorporating the sequence component would trigger changes in cerebellar excitability, but not M1 LTP-like plasticity.

Participants learned the sensorimotor map

Twenty healthy participants (23.8 ± 4.6 years, 12 females) were randomly assigned to either practice a consistent logarithmic sensorimotor map (*Training Group*; $n=10$) or to train on a task that presented different force-cursor maps on each trial (i.e. logarithmic, exponential, sigmoidal, double sigmoidal and linear; *Random Group*; $n=10$; Figure 4.1c). This control task allows matching performance efforts while preventing clear accumulation of knowledge about the sensorimotor map.

Participants in the Training group practiced the logarithmic map for three days, while the random group, to prevent boredom and frustration, only practiced one day. To assess the knowledge of the sensorimotor maps, we quantified the average time to hit each target from the “go” cue (movement time) and error-rate (proportion of trials in which endpoint cursor position was outside target boundary) for each block of 30 trials. We compared the differences in movement time and errors across the Training and Random groups where performance was matched on Day 1 (i.e. sum of the difference between Blocks 1 and 5). We found that only the Training group decreased their movement time ($t(18) = 5.02$; $p < 0.001$), while both groups maintained consistent error-rates throughout training ($t(18) = 0.51$; $p = 0.307$).

In the training group we found a significant reduction in movement time ($F(6,72) = 13.26$; $p < 0.001$), while error-rate remained consistent throughout the training sessions ($F(6,72) = 0.56$; $p = 0.592$; Figure 4.2a&b). This effect was driven by performance improvement in Day 1 ($p = 0.007$) and Day 2 ($p = 0.005$) relative to Day 3, indicating greater sensorimotor map learning within the first two training sessions. Together, these results indicate that only Training Group individuals learned the logarithmic map.

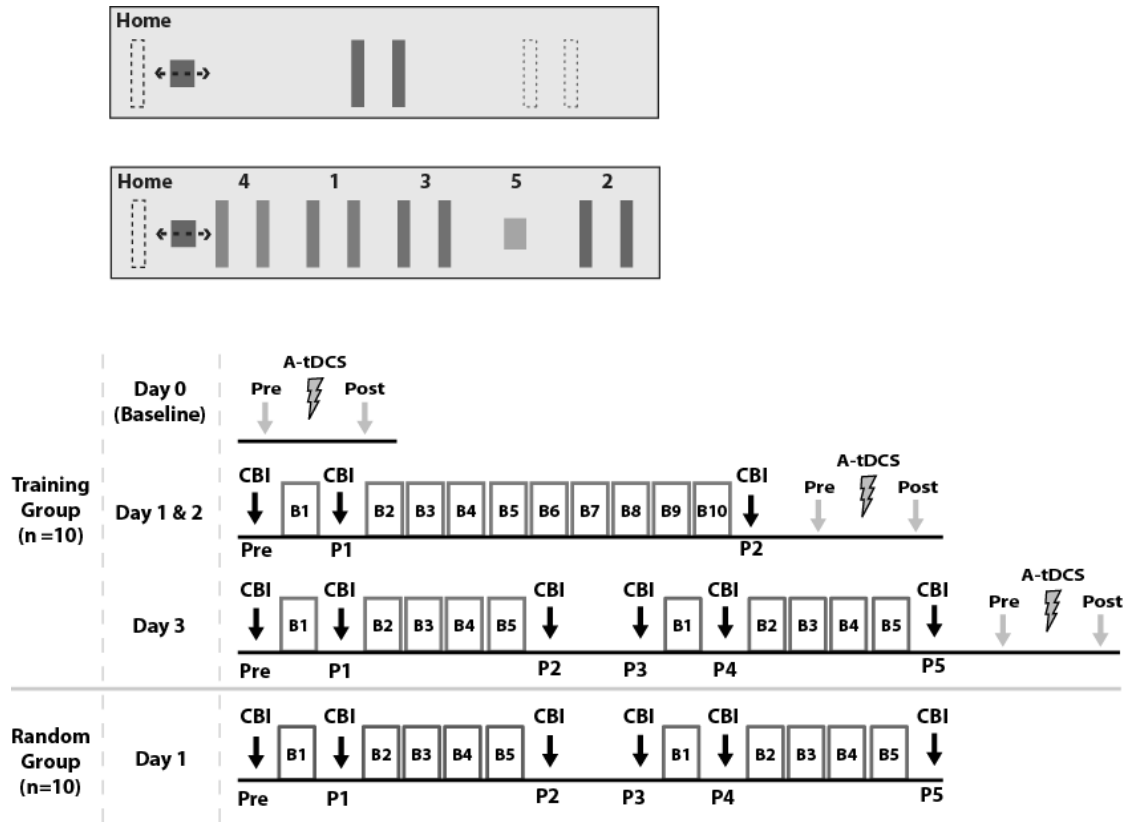


Figure 4.1: Experiment 1 Protocol. (A-B) Depiction of the visual stimuli used to learn the sensorimotor map (A) and skill tasks (B). (A) During sensorimotor map training, participants controlled the lateral movement of a computer cursor hit an individual target presented on a computer screen (magenta). The position of the target changed on each trial to expose participants to the entire logarithmic mapping between isometric pinch force and cursor displacement (outlined target). (B) During skill learning, participants were instructed to navigate the cursor between home position and five targets. The sequence order consisted of home-1-home-2-home-3-home-4-home-5. (C) Participants were divided into either Training (n=10) or Random (n=10) groups. Only Training group individuals participated in three days of training (Day1; Day2; Day3). On Day1 and Day2, individuals completed ten blocks consisting in 30 trials of sensorimotor map training (blue). Day 3 training began with five blocks of sensorimotor

training, followed by five blocks of skill training (green). Cerebellar excitability measurements (black arrows) for this group were assessed prior to training (Pre) and after the first (P1) and final sensorimotor map training behavioral block (P2). This measure was also assessed in a similar fashion throughout skill training (Day3; P3, P4, P5). Random group individuals (n=10) trained only on one day, where the behavior and cerebellar excitability measure was identical to the Training group, except that Random group individuals performed in a randomized version of the sensorimotor map task (red). For the Training group, MEP amplitudes (black arrows) were measured before and after application of A-tDCS (grey ray) to assess M1 LTP-like plasticity. This was measured on a day where no training occurred (Day0) and after each training session (Day1, Day2 Day3).

Knowledge of the sensorimotor map facilitate skill learning

We predicted that the Training group would perform better at a skill task that involves learning a logarithmic map besides a sequence of movements. To measure this, we quantified changes in the speed-accuracy tradeoff function (i.e. Skill measure) for each training block when subjects practice the SVIPT task (Reis et al. 2009; Cantarero et al 2013 a&b; Wymbs et al. 2016). As expected, we found that the training group performed the task better from the onset and throughout training when compared to the random group (Training and Random groups skill measure interaction: $F(4,72) = 2.98$; $p = 0.024$; posthoc Block 1; $p < 0.001$; Figure 4.2c), meaning that subjects exposed to the random maps were not able to catch up with the other group. These results indicate that knowing the sensorimotor map facilitated skill learning.

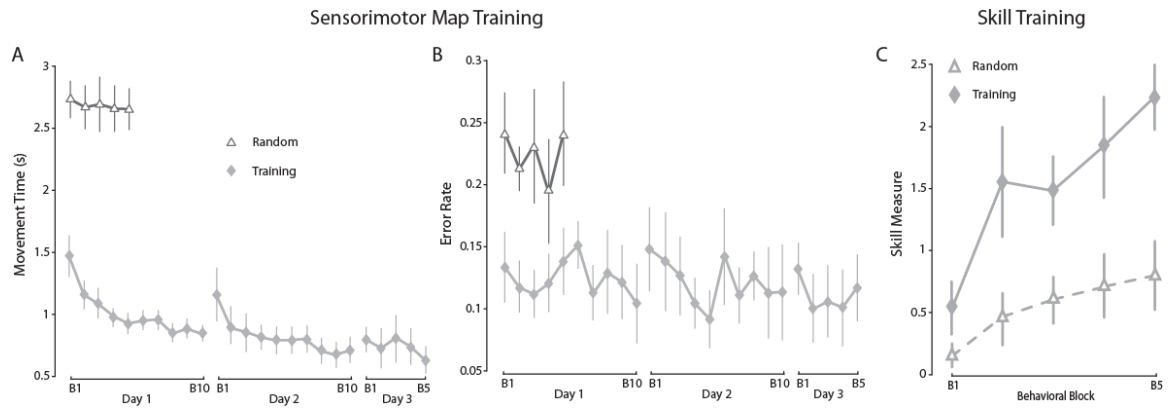


Figure 4.2: Training on the sensorimotor map led to better skill learning. (A-B) Sensorimotor Map Training Results for the Training (blue) and Random (red) groups. The X-axis depicts training blocks (average of 30 trials) and the y-axis represents the average movement time (A) and average error rate (B). Vertical grey solid lines represent the separation between training days and the data represent the mean \pm SEM for each block. (C) Skill Training Results for the Training (solid-green) and Random (dashed-green) groups. The average skill measure is plotted on the y-axis across the behavioral blocks. Note, Training group participants acquisition and performance of the skill task was superior to the Random group.

Cerebellar excitability changes occur during both map and skill learning

To investigate physiological changes in the cerebellum, we used a paired-pulse TMS technique to probe the strength of cerebellar connectivity to M1. Applying TMS to the cerebellum results in subsequent inhibition of contralateral M1, an effect known as cerebellar inhibition (CBI; Ugawa et al., 1995; Pinto and Chen, 2001; Daskalakis et al., 2004; Celnik 2015). We have previously shown that the magnitude of CBI is modulated when learning error-based motor tasks in the absence of M1 excitability changes (Jayaram et al., 2011; Schlerf et al. 2012, 2014). In other words, changes in this physiological measure is thought to reflect modulation of the inhibitory

output the cerebellar cortex exerts to the deep cerebellar nuclei, which in turn has a disynaptic excitatory connection to M1 via the thalamus.

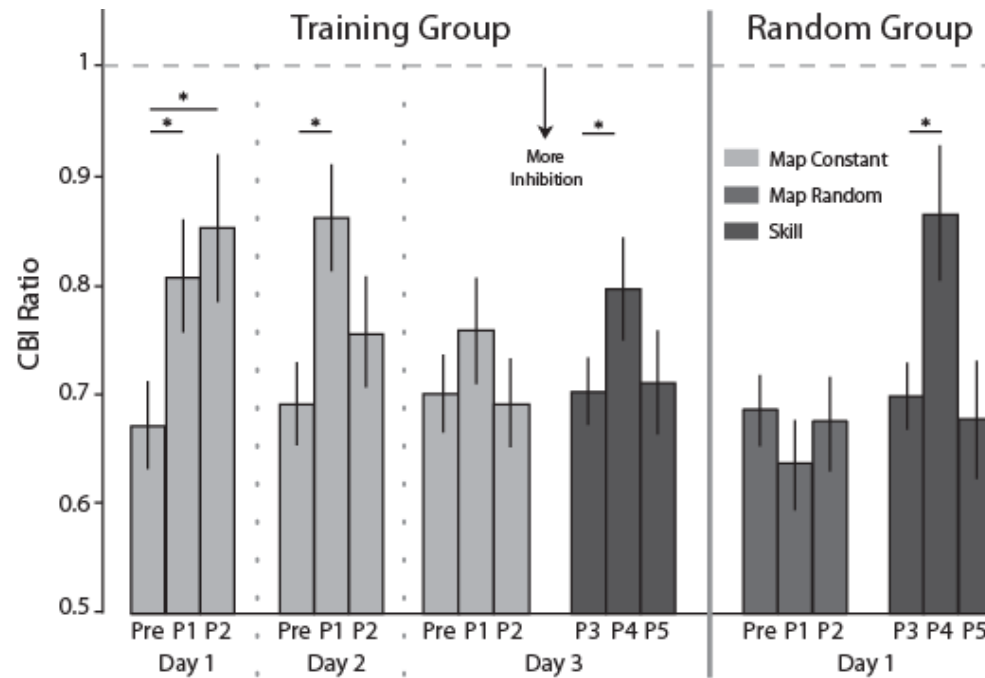


Figure 4.3: Cerebellar excitability changes when learning the sensorimotor map and skill task. Bar graphs and vertical error bars depict the mean \pm SEM of the CBI ratio at different stimulation time-points. Dashed horizontal line depicts the normalized unconditioned MEP amplitude and the dashed vertical line represents the separation between training days. Solid grey vertical line separates the results from each group. The CBI ratio increases (i.e. less inhibition) only for the Training group (blue) and not for the Random group (red) throughout sensorimotor map training. On the other hand, CBI reduced early for both Training and Random groups (green) when learning the skill task.

When assessing cerebellar excitability in the Training group prior to, during and after each sensorimotor map training sessions, as well as during the skill training blocks (Figure 4.1), we found changes in CBI varied across training sessions (Training Group CBI session x time interaction: $F(6,72) = 2.32$; $p=0.042$; Figure 4.3). Specifically, during sensorimotor map training, we found a reduction of CBI throughout Day1 (P1, $p = 0.020$; P2, $p < 0.01$), early Day2 (P1, $p < 0.01$), but not in Day3 (all $p > 0.2$). This indicates that as participants learn the maps over days, cerebellar excitability was modulated early on in Day 1 and 2. On Day 3, when learning was less, CBI did not change. Furthermore CBI was also modulated early during motor skill learning (P3; $p=0.04$), suggesting that cerebellar excitability changes also occur when learning a sequence of actions on the trained sensorimotor map.

We additionally determined CBI changes in the Random group during sensorimotor map and skill task training. We found only a significant change in CBI early during skill training when the sensorimotor map was held constant, but no changes when participants trained the random presentation of many different sensorimotor maps (Random group CBI session interaction: $F(2,36) = 2.32$; $p=0.023$; posthoc P3; $p < 0.01$). These results are consistent with prior findings indicating that CBI changes are specific to learning and not due to motor performance (Jayaram et al., 2011; Schlerf et al., 2012; Spampinato and Celnik, 2017).

M1 LTP-like plasticity was evident only after sequential-skill learning

To assess M1 LTP-like plasticity following the sensorimotor map and skill learning, we used a previously described protocol that combines anodal transcranial direct current stimulation (AtDCS) and transcranial magnetic stimulation (TMS; Cantarero et al., 2013 a&b; Spampinato and Celnik, 2017). Briefly, we applied AtDCS after each training session (Baseline, Day1, Day2, Day3) and measured with TMS MEP amplitudes to determine the presence of M1 excitability

potentiating aftereffects (see methods section for details). The potentiating effects of AtDCS reflect a mechanism similar to LTP, as they are dependent on NMDA receptor activity (Liebetanz et al., 2002; Nitsche et al., 2003; Nitsche et al., 2004; Fristch et al., 2010). Thus, if learning a specific task is associated with M1 LTP-like mechanisms then AtDCS aftereffect on M1 excitability should be occluded (Cantarero et al., 2013 a&b; Spampinato and Celnik, 2017). When we evaluated the presence of occlusion after each session, we found significant differences on M1 excitability across training sessions ($F(3,36) = 3.57$; $p = 0.027$; Figure 4.4). The AtDCS aftereffects following sensorimotor map training (Day 1 and Day 2) showed similar potentiation effects relative to the baseline responses recorded on a day when participants did not train ($p > 0.90$; $p = 0.494$ respectively). On the other hand, we found occlusion of potentiation effects following skill task training when compared to baseline ($p = 0.019$), a finding consistent with previous studies (Cantarero et al., 2013 a&b; Spampinato and Celnik, 2017). These results indicate that M1 engages LTP-like plastic changes only when a sequence of actions is incorporated, but not when learning the sensorimotor map.

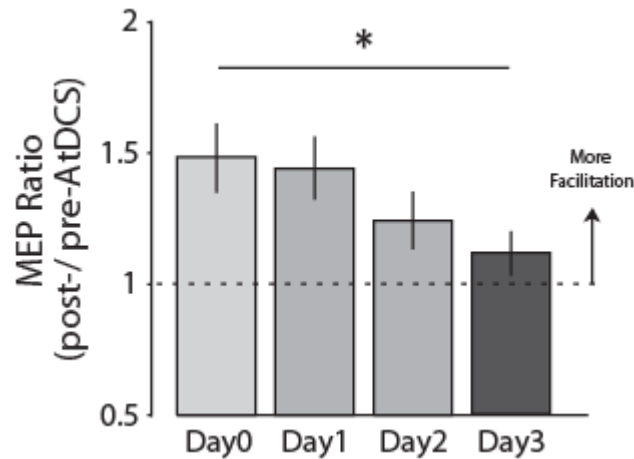


Figure 4.4: *MI LTP-like plasticity is prominent only after skill learning. Bar graphs and vertical error bars depict the mean \pm SEM of the MEP ratio (post-AtDCS MEP amplitudes normalized to pre-AtDCS MEP amplitudes) for the Training group after each session (Day 0, Day 1, Day 2, Day 3). Dashed horizontal lines depict the normalized MEP amplitude of pre-AtDCS (1.0), thus values increasingly larger than 1.0 represent a larger response to AtDCS. Only the responses to AtDCS following skill training (green) and not following sensorimotor map training (blue), were significantly smaller from rest (grey).*

Learning a sequence on a known sensorimotor map, elicits changes in cerebellar excitability and MI LTP-like plasticity

It is possible that cerebellar excitability changes following sequence learning on the sensorimotor map is related to the participants continuing to learn the dynamics of the sensorimotor map.

Alternatively, it is also possible that sequence learning also engages cerebellar plasticity.

Furthermore, if the sequence component of a skill task is driving M1 LTP-like changes, then AtDCS aftereffects following finger sequence learning should also result in occlusion. To disentangle these predictions we assessed the same physiological markers, CBI and M1 LTP-like plasticity, in a second experiment where participants learned a sequence of movements on a computer keyboard, in which the demand to learn a sensorimotor map is minimal (Figure 4.5).

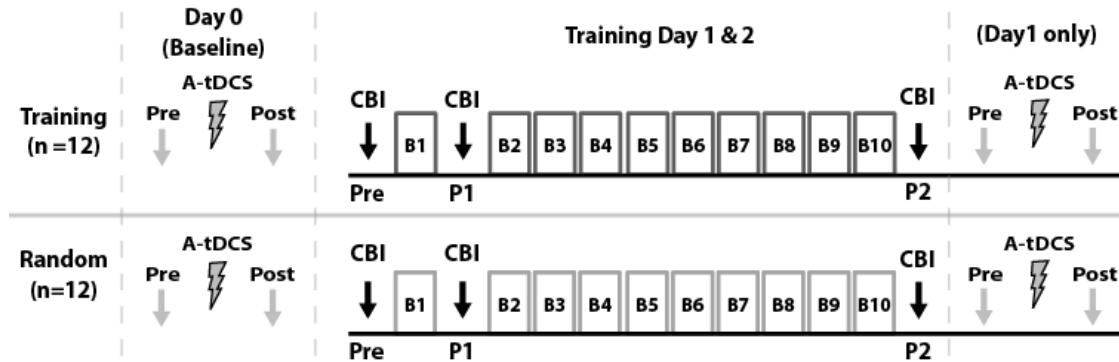


Figure 4.5: Experiment 2 Design: Learning sequence in a well-known sensorimotor map.

Participants from both the Training (teal; $n=12$) and Random (orange; $n=12$) groups trained on motor sequences for two days. Only Training group individuals performed the same nine-element sequence throughout training, whereas the Random group was exposed to a different ordered sequence on each trial. For each group, cerebellar excitability measures were assessed prior to training (Pre), after one block (P1) and at the end of training (P2). Moreover, M1 LTP-like plasticity was assessed on a non-training session (Day 0) and after the first training session (Day1).

Twenty-four healthy participants (24.4 ± 4.1 years, 14 females) completed a two-day experimental session. Participants were randomly assigned to either practice a consistent 9-element sequence involving three neighboring keys (*Training Group*; $n = 12$) or practice on a version of the task where the order of the 9-element sequence changed on each trial (*Random Group*; $n=12$). All participants were instructed to only use their index finger to hit the computer keys and told to perform movements as quickly and accurately as possible. Importantly, the key-specific cues represented visually on a computer screen always remained in the same location (no finger-visual associations), and the sequence was paused if an error occurred and restarted upon the appropriate key press (see methods section for details).

To assess motor sequence learning, we compared group differences in online error-rate, reaction time, and movement time across training sessions. We found that the Training group significantly improved their reaction time and movement time compared to the Random group (movement time interaction: $F(1,22) = 16.35$; $p = 0.001$; error-rate interaction: $F(1,22) = 14.33$; $p = 0.001$; Figure 4.6a&b), while maintaining comparable error-rates (error-rate interaction: $F(1,22) = 1.77$; $p = 0.197$). Critically, the movement time ($t(22)=0.297$; $p = 0.770$) and reaction time ($t(22)=0.544$; $p = 0.592$) across groups were similar in the first five trials, indicating that both groups began at the same performance level.

When we assessed cerebellar excitability before, during and after training across groups, we found a selective reduction of CBI only early during consistent sequence training on Day1 (Training and Random group CBI interaction: $F(2,44) = 3.67$; $p=0.034$; posthoc P1; $p=0.001$; Figure 4.6c). This indicates that motor sequence learning lead to cerebellar excitability changes, independent of acquiring a new sensorimotor map. Moreover, when we evaluated the effects of AtDCS on M1 excitability after training, only the Training group showed significant occlusion of AtDCS potentiating effects relative to baseline responses (Training and Random group interaction: $F(2,44) = 4.36$; $p=0.048$; posthoc Training, $p = 0.007$; Figure 4.6d). Together, these

findings suggest that an interaction of M1 and cerebellar plasticity is necessary to learn a new sequence of movements, even when there is minimal demand to learn a sensorimotor map.

It could be argued that cerebellar excitability changes early in sequence learning are due to changes in training group performance compared to the random group (i.e., faster sequence execution time), independent of sequence learning. To exclude this possibility, we assessed cerebellar excitability changes after when individuals produced rhythmic finger tapping at two frequencies (fast and slow tempo) imposed by an auditory metronome. Here, we tested six individuals from the training group in experiment 2 (24.5 ± 4.93 years, 3 females) in a follow-up session and assessed cerebellar excitability at rest, and after performing rhythmic tapping at each tempo. Importantly, performance of the distinct tempo paces were counterbalanced and reflected the difference of performance within the first sequence training block in experiment 2 (see methods for details). We found no significant differences in CBI ($F(2,10) = 0.27$; $p = 0.737$; Figure 4.6.e), supporting our claim that modulation of cerebellar excitability seen during sequence learning are not due to changes in sequence movement time performance.

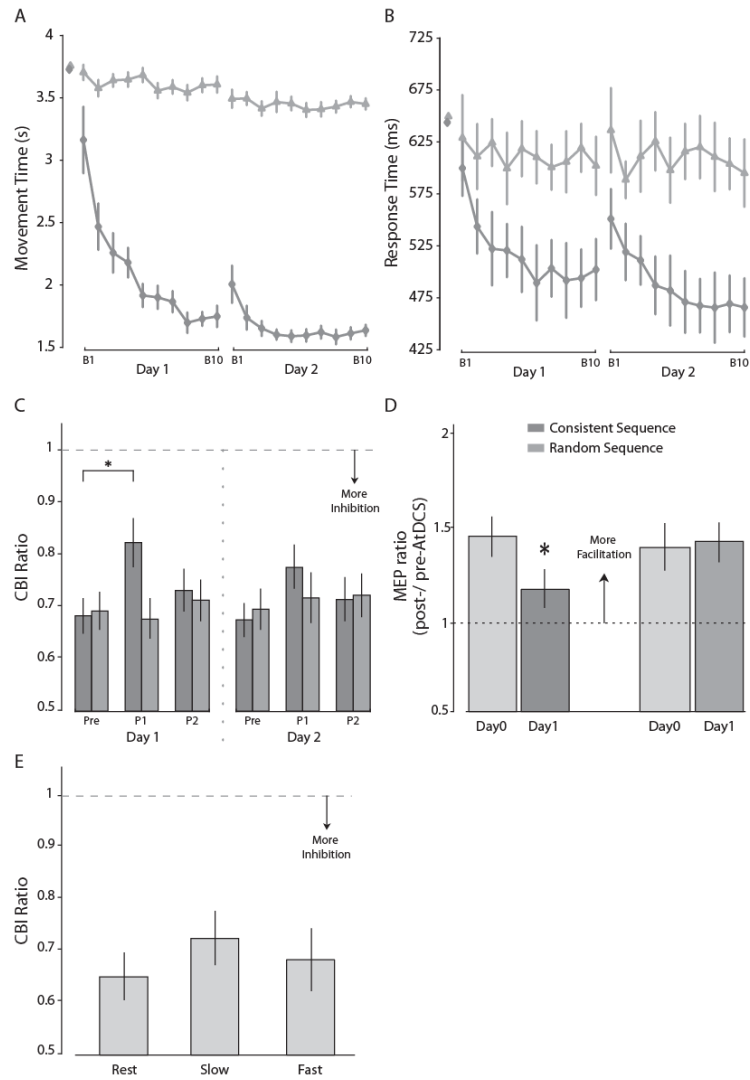


Figure 4.6: Experiment 2 Results: Learning a sequence on a well-known sensorimotor map, elicits changes in cerebellar excitability and M1 LTP-like plasticity. (A-B) Behavioral Results for the Training (teal) and Random (orange) groups. The X-axis depicts training blocks (average of 30 trials) and the y-axis represents the average movement time (A) and average reaction time (B). Vertical grey solid lines represent the separation between training days and the data represent the mean \pm SEM for each block. Note that all individuals started with similar performance, but only the Training group improved their movement and reaction time. (C) Cerebellar excitability changes. Bar graphs and vertical error bars depict the mean \pm SEM of the CBI ratio for the

Training (teal) and Random groups (orange) at each stimulation time-points (Pre, P1, P2). Dashed horizontal line depicts the normalized unconditioned MEP amplitude and the dashed vertical line represents the separation between training days. The CBI ratio changes for the Training group but not for Random group early in each sequence session. (D) Occlusion of M1 LTP-like plasticity. Bar graphs and vertical error bars depict the mean \pm SEM of the MEP ratio (post-AtDCS MEP amplitudes normalized to pre-AtDCS MEP amplitudes) for each group's baseline session (grey) and for the training session (Training group in teal; Random group in orange). Dashed horizontal lines depict the normalized MEP amplitude of pre-AtDCS (1.0). Only the Training group showed significant occlusion of M1 LTP-like plasticity after sequence training when compared to baseline responses. (E) Rhythmic Tapping and cerebellar excitability changes. Bar graphs and vertical error bars depict the mean \pm SEM of the CBI ratio recorded at rest, and following slow-tempo and fast-tempo rhythmic finger tapping. Dashed horizontal line depicts the normalized unconditioned MEP. No differences were found in the CBI ratio for the different conditions.

4.3 Discussion

We assessed distinct physiological markers of the cerebellum and M1 when individuals learned two separate components of a motor skill. Here, we show that acquiring a new sensorimotor map, thought to represent learning of skill task dynamics, involved plastic changes in the cerebellum, but not in M1. On the other hand, learning a sequence of movements entailed plastic changes in both of these brain regions. This indicating that the properties defining distinct motor skill components determine what the relative contributions of different brain regions are when learning a new skill.

Similar to previous findings linking motor adaptation and cerebellar plasticity (Jayaram et al., 2011; Schlerf et al., 2012), we found cerebellar excitability changes (i.e. reduction of CBI) throughout the acquisition of a new sensorimotor map. We suggest that cerebellar-dependent error-based learning, which heavily contributes to motor adaptation, plays a similar role in developing an internal representation of skill task dynamics. We reason that when first interacting with the force-visual map, there is no motor history for an accurate forward model, thus resulting in large sensory prediction. As such, the process of learning involves adjust motor commands on each exposure by refining internal forward models driven by reducing (online and endpoint) sensory prediction errors. We postulated that developing this internal model is a much slower process than recalibrating existing forward models that occurs in motor adaptation; therefore, we had participants train on this mapping for three days and evaluated CBI throughout training. Consistent with this hypothesis, robust cerebellar excitability changes were presented throughout the first training session and early on in second training session. This suggests that as participants become familiar with the mapping rule, the contribution of an error-based mechanism plays a reduced role. Moreover, we also found no evidence for neurophysiological changes in the random group, consistent with our previous results indicating that a large sensory prediction error alone is not sufficient to drive changes in cerebellar excitability if no accumulation of learning occurs

(Schlerf et al., 2012; Spampinato and Celnik, 2017). Thus, we believe the reliance on error-based mechanism is what drives cerebellar excitability changes when learning the dynamics of a new motor skill.

While it is well known that motor learning induces plastic changes in the cerebellum (Anderson et al., 1996; Kleim et al., 1998; Schlerf et al., 2012), how multiple sites of plasticity within the cerebellar cortex contribute to learning a new skill remain largely unknown. Among the many sites and forms of synaptic plasticity involved in learning (D'Angelo et al., 2016), long-term depression (LTD) of parallel fiber-Purkinje cell synapses remain one of the most influential in interpreting the reduced CBI found during sensorimotor map learning. For instance, studies in non-human primates have shown cerebellar-dependent motor learning is associated with LTD of parallel fiber-Purkinje cell synapse (Medina and Lisberger, 2008; Yang and Lisberger 2013), driven by the duration of climbing fiber inputs relaying movement error signals (Yang and Lisberger, 2014). Thus, if Purkinje cell activity is reduced due to the learning-related LTD, then applying a cerebellar TMS conditioning pulse with the same intensity prior to learning will lead to less activation of the cerebello-dentato-thalamo-cortical pathway, resulting in a release of inhibition of M1 triggered by a subsequent test pulse (Celnik 2015). We think the current results suggest that an LTD-like mechanism underlies the error-based cerebellar contribution to learning a new sensorimotor mapping.

On the other hand, we found no evidence for M1 LTP like plasticity during sensorimotor map learning. We interpret that this finding is due to the higher reliance on other brain regions and their associated forms of learning (i.e. cerebellum/prefrontal cortex and error-based/cognitive) to acquire this component. Indeed, several studies utilizing non-invasive brain stimulation over M1 did not modulate the acquisition rate of motor adaptation (Richardson et al., 2006; Hadipour-Niktarash et al., 2007; Gaeta et al., 2011; Orban de Xivry et al., 2011 a&b; Herzfeld et al., 2014) where the contribution of error-based learning are critical. Instead, disruptive TMS over M1 only

impairs motor adaptation when the same movements are repeated (Orban de Xivry et al., 2011), consistent with neurophysiological evidence demonstrating robust changes in M1 activity late in adaptation (Paz et al., 2003; Paz and Vaadia, 2004; Richardson et al., 2012). Indeed, it has been argued that movement repetition elicits a different form of memory than those acquired from error-based learning (Diedrichsen et al., 2010; Huang et al., 2011). However, there is substantial evidence that has indicated that M1 plays a critical role in retention of motor learning (Muellbacher et al., 2002; Robertson et al., 2004; Haipour-Niktarash et al., 2007; Reis et al., 2009; Galea et al., 2011). Thus, one might expect that occlusion of M1 LTP-like plasticity, a mechanism linked to motor retention (Cantarero et al., 2013; Spampinato and Celnik, 2017), would occur following map training since participants displayed better map performance at the start of the second and third training session. However, this may only be true for some motor memories, such learning visuomotor associations and not for learning novel force production rules. For example, some studies have shown retention of a dynamic force-field learning is unaffected by non-invasive stimulation aimed to modulate M1 activity (Baraduc et al., 2004; Herzfeld et al., 2014) and instead may be encoded in regions outside of M1, such as the cerebellum (Lu et al., 1998; Li Voti et al., 2014; Herzfeld et al., 2014; Wessel et al., 2015). Therefore, we suggest that the nature of the sensorimotor map task studied here (i.e. exposing participants to the entire task space) may explain why no changes in M1 LTP-like plasticity occurred.

Our observation that cerebellar excitability changes with motor sequence learning is consistent with previous findings from patient (Pascual-Leone et al., 1995; Molinari et al., 1997; Gomez-Beldarrian et al., 1998; Shin and Ivry 2003; Spencer and Ivry, 2009), imaging (Doyon et al., 2002; Penhune and Doyon, 2005; Seidler et al., 2005; Grafton et al., 2008; Steele and Penhune, 2010), brain stimulation (Torriero et al., 2007, 2011; Ferrucci et al., 2013), and animal lesion studies (Passingham and Nixon, 2001). It has recently been suggested that the role of the

cerebellum in sequence learning is to acquire the optimal internal model (i.e. velocity, force, timing, etc.) for sequence performance (Penhune and Steele, 2012). While this may explain why cerebellar excitability changes occurred only early-on in learning the sequential components of experiment 1 and 2 compared to the pronounced effects observed throughout sensorimotor map training, we and others believe that an error-based form of learning likely makes a minimal contribution to learning sequences (Spencer and Ivry, 2009; Stark-Inbar Alit et al., 2016), as it does not involve a change in task dynamics (i.e. no sensorimotor map calibration) nor on vector error information. Instead, learning entails formation of associations or predictions between successive sequence elements and errors committed in sequence performance reflect selecting an inappropriate action (i.e. target/sequence element prediction error). Given that the activity in overlapping cerebellar regions has been shown to respond to different types of errors (Diedrichsen et al., 2005), the changes seen during sequence learning may reflect a process beyond sensory prediction errors.

Recent anatomical studies have revealed reciprocal connections between the cerebellum and prefrontal cortex (Kelly and Strick, 2003), as well as cerebellum and basal ganglia (Bostan and Strick, 2010), supporting the possibility that these brain regions and their associated forms of learning (i.e. strategy and reward-base) may influence cerebellar plasticity. For example, a recent study suggested that reinforcement signals from the basal ganglia might prime the cerebellar sensitivity to incoming error signals (Galea et al., 2015), thus one interpretation is that cerebellar excitability modulation reflects these regions interactions to anticipating associations between distinct elements of a sequence. Alternatively, cerebellar-prefrontal loops may contribute to a working memory process establishing stimulus-response representations (Spencer and Ivry, 2009; Bo et al., 2011). Our results from Experiment 2 suggest an additional role beyond learning stimulus response-associations, as participant groups exposed to consistent or random sequences relied on the same, low-demanding stimulus-response association (i.e. computer screen cue directly reflected a keyboard key). Instead, one possibility for the changes in cerebellar

excitability may relate to this network shifting behavioral performance from an unpracticed and attention demanding state into a more skilled and automatic one (Jenkins et al., 1994; Doyon et al., 1998; Thach, 1998, Lang and Bastian 2002; Wu et al., 2004; Boyd and Lang 2008). Future work will need to address how these regions interact when learning new motor skills.

We also found that M1 LTP-like plasticity occurs after motor sequence learning, but not following sensorimotor map learning. We expected that the repetitive nature of motor sequence learning would elicit occlusion of M1 LTP-like plasticity following training. This is consistent with prior animal and human work linking this physiological phenomenon with repetitive and reinforcement motor learning and retention of motor memory (Rioul-Pedotti et al., 1998, 2000; Muellbacher et al., 2002; Rosenkranz et al., 2007; Molina-Luna et al., 2009; Hosp et al., 2011; Cantarero et al., 2013a&b; Spampinato and Celnik, 2017). We interpret our results to reflect the engagement of these forms of learning contribution towards encoding a control policy for the learned sequence of movements in M1. This is consistent with animal work showing that M1 encodes spatial-temporal parameters of sequences (Carpenter et al., 1999; Matsuzaka et al., 2007) and that motor sequence learning is associated with the expansion of M1 output maps (Pacual-Leone et al. 1995; Classen et al. 1998), via LTP/LTD-induced synaptic strengthening of horizontal connections (Sanes and Donahoue 2000; Pedotti et al., 1998, 2000). Since modifying synaptic efficacy is activity-dependent and repetitive motor learning activates LTP-like mechanisms, our results indicate M1 contributes to motor sequence learning by storing sequence representations reliant on repetition and reinforcement of the same movement pattern.

In conclusion, we show that learning a new sensorimotor mapping of a skill task leads to changes in cerebellar excitability but not to M1 LTP-like plasticity changes; whereas learning a new sequence of movements in a well-trained map elicits changes in both cerebellar excitability and M1 LTP-like plasticity. These findings indicate that learning complex motor skills involve a

harmonious blend of distinct physiological mechanisms and their associated neural substrates in order to successfully learn different motor components of a skill (i.e. how to interact with a new device/environment, planning of movement sequences, etc.). Our results may explain why the nature of a motor task being learned can yield different outcomes when targeting specific brain regions with non-invasive brain stimulation (Stagg et al., 2011; Kantak et al., 2012; Saucedo-Marquez et al., 2013; Kwan et al., 2015), and further suggest that targeting one region to augment motor learning may yield limited benefits. We believe this has important implications towards developing rehabilitation strategies aimed at enhancing motor recovery following cerebral-stroke. For instance, beyond focusing on lesioned cortical regions, targeting additional brain regions, as such as cerebellum, may enhance motor recovery given its role adjusting movements to altered environments and learning rule-based movement patterns.

4.4 Materials and Methods

We recruited a total of 44 naïve healthy right-handed individuals (mean age = 24.11 ± 4.36 years; 26 female) with no history of neurological disorders. Exclusion criteria included the use of alcohol, recreational drug use and prescribed medication affecting the central nervous system, all of which may alter plasticity and motor learning. This study was approved by the Johns Hopkins University School of Medicine Institutional Review Board. All participants provided written informed consent before participating in the study

Neurophysiological assessments: Transcranial magnetic stimulation (TMS)

We used a 70 mm-diameter figure-of-eight TMS coil (Magstim 200²) over the left M1 to elicit motor evoked potential (MEP) of the first dorsal interosseous (FDI) muscle of the right hand. We used a neuro-navigation system (BrainSight; Rogue Research) to ensure stimulation location over the desired M1 location occurred stayed consistent from session to session. To do this, we

identified and registered a “hot spot” with the best representation of the right FDI muscle. MEPs were recorded with electromyographic (EMG) activity via disposable surface electrodes placed over the FDI muscle. EMG signals were sampled at 2 kHz, amplified at 1 kHz and band-pass filtered (10–500 Hz) using an amplifier (Octopus AMT 8; Bortec Biomedical, Alberta, Canada) and data acquisition software (Signal 4.02; CED, Cambridge, England). The data was stored and analyzed off-line on another computer using custom Matlab scripts (MathWorks, MA, USA). For all analysis, we used peak-to-peak MEP amplitudes as the index of M1 excitability.

Measures of Cerebellar Excitability (CBI)

To determine cerebellar excitability changes, we delivered TMS using a double-cone coil (110mm mean diameter, Magsitm) placed over the cerebellar cortex ipsilateral to the trained hand and 3cm lateral to the inion, with the stimulator current directed downward. Similar to previous studies, we assessed CBI by delivering a TMS conditioning stimulus (CS) over the cerebellar cortex 5 ms prior to test stimulus (TS) over the contralateral motor cortex (Ugawa et al., 1995; Pinto and Chen, 2001, Daskalakis et al., 2014). We delivered 10 CS + TS stimuli to measure CBI along with 10 unconditioned TS stimuli in a random order. CBI was quantified as the ratio of mean MEP amplitude in the CS+TS relative to TS. To avoid antidromic stimulation of the pyramidal tract with the cerebellar CS, the intensity for cerebellar stimulation was set at 5% below the brainstem active motor threshold (aMT; Werhahn et al., 1996; Galea et al., 2009). To do this, the double-cone coil was placed over the inion as subjects pre-activated their FDI muscle. Threshold was defined as the nearest 5% stimulator output that elicited an MEP of 50 μ V in the pre-activated FDI muscle in 5 of 10 trials. The intensity of cerebellar CS was then set at 5% less than the brainstem threshold, or if the threshold was not found at 75% of stimulator output, we used 70% stimulator output. Throughout each stimulation time point, the intensity of the TS was adjusted to evoke an MEP of \sim 1Mv.

Quantifying Anodal Transcranial Direct Current Stimulation (AtDCS) Aftereffects

To assess corticomotor excitability, we first determined the stimulus intensity needed to evoke an MEP with peak-to-peak amplitude of ~ 1 mV (S1mV). We recorded 10 MEPs at this intensity prior to applying AtDCS. Using a Chattanooga Ionto Phoresor II Auto device (model PM850; IOMED, UT, USA), we then delivered AtDCS through two 25 cm² sponge electrodes soaked in a saline solution for 7 minutes at an intensity of 1 mA. Electrodes were placed over the left M1 “hot spot” for the FDI muscle and right supra-orbital area. Following the AtDCS application, we recorded 10 MEPs at S1mV intensity immediately after cessation and repeated this recording every 5 minutes until 30 minutes post AtDCS application. To assess AtDCS aftereffects, we averaged the recorded 10 MEP amplitudes at each time point and normalized these values to the average of 10 MEP amplitudes recorded prior to AtDCS application.

To quantify learning-related occlusion of M1 LTP-like plasticity, for each subject we compared the AtDCS aftereffects following motor performance compared to their baseline AtDCS response (i.e. day where no motor performance was completed). To do this, we calculated the grand mean normalized post-AtDCS MEP amplitudes for each session. We then determined and classified occlusion of M1 LTP-like plasticity changes when we the post-AtDCS MEP amplitudes after motor training were smaller when compared to the baseline session.

We also pre-screened for non-responders to AtDCS based on post-AtDCS MEP changes from a baseline session. A non-responder was classified when the mean of normalized MEP amplitude across each post-AtDCS time points did not exceed 1.0 (i.e., smaller than pre-AtDCS MEP). Six individuals did not meet this criterion and were therefore excluded from the study.

Experimental procedure: Experiment 1

Sensorimotor Map Task

Participants were seated in front of a vertical computer screen, in which they controlled a cursor by applying a pinch force with the right thumb and index finger to a force transducer. The overall goal was to move the cursor between the start position and a target presented on the screen. Target location was randomized trial-by-trial to encourage participants to explore along the entire task (monitor) space. Training group participants experienced a consistent logarithmic force-cursor mapping between pinch-force production and on-screen cursor movement. On the other hand, Random group individuals were exposed to varying force-distance maps (i.e. logarithmic, exponential, sigmoidal, double sigmoidal and linear) on each trial. For both groups, the target was presented on the screen simultaneously with an instruction cue (green circle) indicating the start of the trial. All participants were encouraged to accurately move to the target as fast as possible. Movement time was considered as the amount of time from movement onset until the target was reached. An accurate movement was considered only if individuals landed the cursor endpoint within the target boundaries. As such, we calculated errors in a binary fashion; individuals either hit or missed the target when attempting to reach the single.

Sequence integration: Sequential Visual Isometric Pinch Task (SVIPT)

After the sensorimotor map training was completed, participants trained on the previously described SVIPT (Reis et al., 2009; Cantarero et al., 2013a&b; Wymbs et al., 2016; Spampinato and Celnik, 2017). Participants used the same force transducer and task space as in the sensorimotor map task. However, here participants were instructed to move the cursor as fast and accurate as possible between a HOME position and 5 targets (the sequence of movements: HOME-1-HOME-2-HOME-3-HOME-4-HOME-5). Both Training and Random groups were exposed to a consistent logarithmic force-distance mapping. The target positions and sequential order was held also consistent throughout training. Similar to the sensorimotor map training, movement time was considered from movement onset until the final target was reached. Trials were only considered successful if participants to hit the targets in the correct order. Again, trial

accuracy was calculated in a binary fashion (i.e. no error or error), regardless of participants committing more than one error.

As previously done (Reis et al., 2009; Cantarero et al., 2013, a&b; Wymbs et al., 2016, Spampinato and Celnik, 2017), we assessed skill learning by quantifying changes in movement speed-accuracy tradeoff function (SAF) for each block. To estimate SAF changes throughout training, we calculated a skill score with the following equation:

$$Skill\ Score = \frac{1 - \text{error rate}}{\text{error rate}(\ln(\text{movement time})^b)}$$

Where error rate and movement time correspond to the averages over each training block (30 trials) and the value of constant b is 5.424 (ref). In addition, we administered each calculated error rate with at least 1 over or undershooting movement.

Experimental procedure: Experiment 2

DSP

Participants were seated in front of a computer screen and performed a 9-element discrete sequence production (DSP) in which participants generated responses to sequential, visually presented cues by using their index finger to hit a desktop computer keys. Sequence targets were displayed using a horizontal display of three square stimuli, representing a direct left to right mapping of three neighboring keys (‘V’ leftmost, ‘B’ middle, ‘N’ rightmost). A highlighted square in blue served as an instruction cue for which key participants had to press. Critically, only once the correct response was made, the next target in the sequence became immediately highlighted. As such, if an incorrect key response was selected, the sequence was paused and only

resumed following the appropriate key response. Each sequence trial started with the presentation of a “go” cue, followed by the onset of the initial DSP stimulus (thick square outlined in blue).

The Training group was exposed to the same 9-element sequence throughout training, whereas the Random group received a randomized 9-element sequence order on each trial. Participants were informed to respond and perform the sequence as fast and accurate as possible. We recorded and calculated the mean cue response time to the first element, movement time, and error-rate for each training block of 30 trials. Response time was considered the amount of time participants needed to select the first highlighted sequence element, whereas movement time was considered from the first element response until the final element response was made. A trial was considered successful only if the entire 9-element sequence was completed without an error (i.e. no error or error), regardless if multiple errors were committed.

Rhythmic Tapping

Six subjects from experiment 2 (24.5 ± 4.93 years, 3 females) participated in a follow-up study where we evaluated cerebellar excitability effects following the production of rhythmic finger at an interval specified by an auditory metronome. Movements required flexion-extension of the index finger, where a complete movement ‘tap’ was considered when the flexed-finger contacted the surface of a table. We had participants tap under a slow tempo (200 BPM) in one performance block and at a fast tempo (135 BPM) in another performance block. This pace mimicked the early differences in sequence performance (i.e. first and last five trials of Block 1) found in experiment 2. The order the different tempo-performance blocks (consisting of 30 trials of nine consecutive ‘taps’) was counterbalanced across the participants. The metronome tone was played at the start of each frequency block and remained present throughout performance. Participants were instructed to produce movements when the metronome rate was internalized.

Data Analysis

We used SPSS (IBM; Version 20) to complete all statistical data analysis. All data are represented as means \pm SEM and effects were considered significant if $p \leq 0.05$. To determine whether the groups in experiment 1 were significantly different in sensorimotor map training, we used two-tailed t-tests to assess online performance differences (i.e. movement time and error-rate). We used the same outcome measures by contrasting performance across the training group's sensorimotor map sessions (i.e. Day1, Day2, Day3). Here, we carried out a one-way repeated measures of ANOVA (ANOVA_{RM}) such that training sessions (MapD1, MapD2, MapD3) served as the between-subjects factor. To assess the performance of the SVIPT, we assess differences in the skill measure by using mixed-effect ANOVA_{RM} with GROUP (training, random) as the between and time (Block1, Block2...Block5) as within-factors. For experiment 2, we assessed sequence learning by comparing online differences in error-rate, reaction time, and movement time in three separate mixed-effect ANOVA_{RM} with between-subject factor for group (training, random) and within-subject factor for session (Day1, Day2).

We used separate mixed-effect repeated measures of ANOVA (ANOVA_{RM}) for all collected physiological measures. Mauchly's sphericity was used to validate normality in each ANOVA_{RM} conducted. When significant differences were identified, we used Bonferroni-Holm corrected *post hoc* analysis was done to account for multiple comparisons. To determine changes in CBI in experiment 1 for the training group, we used ANOVA_{RM} with the between-subject factor for session (MapD1, MapD2, MapD3, Skill) and within-subject factor for time (Pre, P1, P2). The same statistical analysis was applied for CBI changes for the random group, with between-subject factor for session (MapD1, Skill) and within-subject factor for time (Pre, P1, P2). For experiment 2, we performed a mixed-effect ANOVA_{RM} with between-subject factor for group (training, random) and within-subject factor for time (Pre, P1, P2). To assess M1 LTP-like plasticity changes following training, we first calculated the AtDCS potentiation effect for each experimental session. We then calculated the grand mean of normalized MEP amplitude

across post-AtDCS time points. Specifically for experiment 1, we compared these values for the training group by using a one-way ANOVA with within-subject factor for session (MapD1, MapD2, Skill). For experiment 2, we performed a mixed-effect ANOVARM with the between-subject factor for group (training, random) and within-subject factor for session (Day 0, Day 1).

Chapter 5 – General Conclusions

This dissertation presents a series of experimental studies conducted with the goal of understanding cerebellar and M1 physiological mechanisms underlying distinct types of motor learning, in order to better characterize how each mechanism contributes to learning a new behavior. Our results have broad implications that include not only providing a better understanding for the role of specific brain regions towards acquiring different types of motor learning, but also can provide a basis for devising effective rehabilitation strategies for recovery following illness or brain disease.

Are changes in cerebellar excitability (CBI) somatotopy-specific in the presence or absence of adaptive motor learning? It is well known that the cerebellum is critically important for error-based adaptive motor learning, as evidenced by patients with cerebellar damage impairment to account for predictable systematic perturbations. While it was previously shown that cerebellar excitability is modulated (i.e. reduced CBI) during learning (Jayaram et al., 2011; Schlerf et al., 2012), it was unknown whether this effect was reflective of global cerebellar plastic changes or not. Our results extend this knowledge by showing that adaptive learning performed with the hand, is capable of producing changes in CBI for a muscle representation not involved in the task. Of note, we also found learning transferred between the trained and non-trained muscle. Thus, we argue that this explains why the physiological response changed for both muscle representations, indicating that the non-specific effect we found is likely linked to transfer of learning. To disentangle whether modulation of CBI for a non-trained muscle is related to interlimb transfer (i.e. follows learning or transfer of learning) or is a non-specific response, we also investigated changes in cerebellar excitability in the context of movement preparation, where no learning occurred. Here we found that CBI changes only for the muscle involved in movement preparation, indicating that learning-related changes in cerebellar excitability are reflective of a

somatotopy-specific interaction. This has important implications for the interpretation of cerebellar TMS results in the context of learning different behaviors and provides evidence that cerebellar excitability can be probed in motor control studies where no learning occurs. For example, the technique described in this thesis could be utilized to investigate physiological changes in healthy individuals or patients in order to better understand the role of the cerebellum in movement preparation.

What are the temporal physiological contributions of cerebellar and M1 networks during skill learning? Since the cerebellum is engaged in error-based adaptive learning by recalibrating movement dynamics to meet predictable demands, an important question we asked was if a similar mechanism also contributes to acquiring motor skill learning in a similar fashion. Moreover, given the substantial evidence from both animal and human studies showing that repetitive motor learning results in LTP-like plasticity (Rioult-Pedotti et al., 1998; Monfils and Teskey, 2004; Cantarero et al., 2013 a&b) and results in occlusion of further induction of further LTP-like plasticity (Rioult-Pedotti et al., 1999, 2000, 2007; Ziemann et al., 2004; Stefan et al., 2006; Rosenkranz et al., 2007; Cantarero et al., 2013 a&b), we reasoned that contributions from this region would be engaged following significant repetition and reinforcement of the skill.

In chapter 3, we sought to assess neurophysiological mechanisms in the CB and M1 of humans during early and late skill learning using TMS and AtDCS to understand the temporal contributions of these brain regions throughout the different stages of learning a new skill. We demonstrated that changes in CBI occurred early in motor skill, but not late. Interestingly, we found a relationship between skill improvement and early changes in CBI. In other words, participants who showed more CBI changes were better at acquiring the skill. On the other hand, occlusion of M1 LTP-like plasticity occurred only during late, but not early skill training. Together, we interpret these results to suggest that early skill learning relies more on an error-dependent form of learning to potentially acquire the dynamics of the skill task, before other

mechanisms are engaged to acquire and encode specific movements. In support of the latter statement, we also found that participants who showed more occlusion of M1 LTP-like plasticity following their first training session, had better skill retention on the following. As suggested previously (Cantarero et al., 2013a&b), our results provide further evidence that occlusion of M1 LTP-like plasticity is a critical mechanism of skill retention. Overall these results hint towards an important temporal interaction between the cerebellum and M1 in motor skill learning.

While examining the role and interactions of distinct brain regions throughout the different stages of motor skill learning is of great relevance for devising future rehabilitation strategies, we acknowledged that the results Chapter 3 could be related to learning specific aspects of the motor skill task participants were asked to learn. Indeed, the skill task studied in this thesis involves both learning to acquire an understanding of how to generate movements of a computer cursor in a novel environment (sensorimotor map), while performing a specific series of movements (sequence). This idea motivated the work done in Chapter 4, in order to better understand the specific contributions of the cerebellum and M1 in skill learning.

Does learning different components of a motor skill change the weights of cerebellar and M1 contributions? Due to the potential confound mentioned above, we deconstructed the skill task and assessed where changes in cerebellar excitability and M1 LTP-like plasticity are related to the distinct motor skill components. Here, we found that changes in CBI, but not in M1 LTP-like plasticity, are linked to learning the sensorimotor map. On the other hand, both changes in CBI and occlusion of M1 LTP-like plasticity were involved in sequence learning. These findings indicate that cerebellar and M1 plasticity changes found in Chapter 3 reflects the involvement of these physiological mechanisms for learning different motor components incorporated in the skill. In other words, the neural correlates of learning and their temporal dynamics vary dramatically depending on the skill being learning.

The finding that modulation of cerebellar excitability and M1 LTP-like plasticity occurs during motor sequence learning is consistent with several lines of work indicating roles for the cerebellum and M1 in acquiring this behavior (for review, see Doyon et al., 2008; Steele and Penhune, 2013). However, we do find the changes in our cerebellar physiological marker, thought to reflect the involvement of error-based learning (Schlerf et al., 2012), interesting given that sequence learned here did not involve a change in task dynamics and was minimally dependent on errors (Spencer and Ivry, 2009; Stark-Inbar Alit et al., 2016). Instead, the sequence acquisition studied here involves associating successive sequential elements (Spencer and Ivry, 2009), and thus may suggest that forms of learning beyond error-based (e.g. strategy and reinforcement learning) may induce changes in cerebellar excitability. Given the recent anatomical evidence revealing reciprocal connections between the cerebellum and prefrontal cortex (Kelly and Strick, 2003), as well as cerebellum and basal ganglia (Bostan and Strick, 2010), future studies can explore this possibility. Moreover, given that prior studies have also indicated M1 plays a critical role in retention of motor learning, including repetitive, adaptation and skill tasks (Muellbacher et al., 2002; Richardson et al., 2006; Cantarero et al., 2013), the lack of M1 LTP-like plasticity changes following sensorimotor map is also a perplexing result. However, given that human and animal studies indicated that dopaminergic projections to M1 help drive neurophysiological plasticity in M1 (Molina-Luna et al., 2009; Kishore et al., 2012), a future experiment could investigate this physiological marker when individuals are provided reinforcement feedback while learning to interact with a new object or environment.

The results presented here have broad implications for improving the efficiency of motor learning and raise an important question for future studies. Is targeting a specific brain region with interventions aimed to augment motor learning an effective strategy? The results from Chapter 4 suggest the nature of the motor task being trained may dictate whether this is an appropriate strategy, and furthermore may explain why some studies targeting specific brain regions with

non-invasive brain stimulation to modulate learning have yielded conflicting results (Stagg et al., 2011; Katak et al., 2012; Saucedo-Marquez et al., 2013; Kwan et al., 2015). On the other hand, we found cerebellar excitability changes across all of the studies examined in this thesis. Interestingly, this is consistent with a recent imaging meta-analysis review of motor learning demonstrating that the right lateral cerebellum was found consistent across a variety of adaptation and motor sequence tasks (Hardwick et al. 2014). Perhaps targeting this region, along with cerebral areas, may provide as an optimal strategy for developing rehabilitation strategies aimed at enhancing motor recovery following cerebral-stroke.

References

Aiba A, Kano M, Chen C, Stanton ME, Fox GD, Herrup K, Zwingman TA, Tonegawa S (1994) Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* 79:377-388.

Albus JS (1971) A theory of cerebellar function. *Mathematical Biosciences* 10:25-61.

Allen GI, Tsukahara N (1974) Cerebrocerebellar communication systems. *Physiological reviews* 54:957-1006.

Allen GI, Gilbert PF, Yin TC (1978) Convergence of cerebral inputs onto dentate neurons in monkey. *Experimental brain research* 32:151-170.

Alonzo A, Brassil J, Taylor JL, Martin D, Loo CK (2012) Daily transcranial direct current stimulation (tDCS) leads to greater increases in cortical excitability than second daily transcranial direct current stimulation. *Brain stimulation* 5:208-213.

Amadi U, Allman C, Johansen-Berg H, Stagg CJ (2015) The Homeostatic Interaction Between Anodal Transcranial Direct Current Stimulation and Motor Learning in Humans is Related to GABAA Activity. *Brain stimulation* 8:898-905.

Anderson BJ, Alcantara AA, Greenough WT (1996) Motor-skill learning: changes in synaptic organization of the rat cerebellar cortex. *Neurobiology of learning and memory* 66:221-229.

Armano S, Rossi P, Taglietti V, D'Angelo E (2000) Long-term potentiation of intrinsic excitability at the mossy fiber-granule cell synapse of rat cerebellum. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 20:5208-5216.

Avanzino L, Gueugneau N, Bisio A, Ruggeri P, Papaxanthis C, Bove M (2015) Motor cortical plasticity induced by motor learning through mental practice. *Frontiers in behavioral neuroscience* 9:105.

Balitsky Thompson AK, Henriques DY (2010) Visuomotor adaptation and intermanual transfer under different viewing conditions. *Experimental brain research* 202:543-552.

Baraduc P, Lang N, Rothwell JC, Wolpert DM (2004) Consolidation of dynamic motor learning is not disrupted by rTMS of primary motor cortex. *Current biology : CB* 14:252-256.

Bastian AJ (2008) Understanding sensorimotor adaptation and learning for rehabilitation. *Current opinion in neurology* 21:628-633.

Battaglia F, Quartarone A, Ghilardi MF, Dattola R, Bagnato S, Rizzo V, Morgante L, Girlanda P (2006) Unilateral cerebellar stroke disrupts movement preparation and motor imagery. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* 117:1009-1016.

Belmeguenai A, Hosy E, Bengtsson F, Pedroarena CM, Piochon C, Teuling E, He Q, Ohtsuki G, De Jeu MT, Elgersma Y, De Zeeuw CI, Jorntell H, Hansel C (2010) Intrinsic plasticity complements long-term potentiation in parallel fiber input gain control in cerebellar Purkinje

cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:13630-13643.

Block H, Celnik P (2013) Stimulating the cerebellum affects visuomotor adaptation but not intermanual transfer of learning. Cerebellum (London, England) 12:781-793.

Bostan AC, Dum RP, Strick PL (2013) Cerebellar networks with the cerebral cortex and basal ganglia. Trends in cognitive sciences 17:241-254.

Boyden ES, Katoh A, Raymond JL (2004) Cerebellum-dependent learning: the role of multiple plasticity mechanisms. Annual review of neuroscience 27:581-609.

Brooks VB (1975) Roles of cerebellum and basal ganglia in initiation and control of movements. The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques 2:265-277.

Buckner RL, Krienen FM, Castellanos A, Diaz JC, Yeo BTT (2011) The organization of the human cerebellum estimated by intrinsic functional connectivity. Journal of neurophysiology 106:2322-2345.

Butefisch CM, Davis BC, Wise SP, Sawaki L, Kopylev L, Classen J, Cohen LG (2000) Mechanisms of use-dependent plasticity in the human motor cortex. Proceedings of the National Academy of Sciences of the United States of America 97:3661-3665.

Cantarero G, Lloyd A, Celnik P (2013a) Reversal of long-term potentiation-like plasticity processes after motor learning disrupts skill retention. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33:12862-12869.

Cantarero G, Tang B, O'Malley R, Salas R, Celnik P (2013b) Motor learning interference is proportional to occlusion of LTP-like plasticity. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33:4634-4641.

Cantarero G, Spampinato D, Reis J, Ajagbe L, Thompson T, Kulkarni K, Celnik P (2015) Cerebellar direct current stimulation enhances on-line motor skill acquisition through an effect on accuracy. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 35:3285-3290.

Carey MR (2011) Synaptic mechanisms of sensorimotor learning in the cerebellum. *Current opinion in neurobiology* 21:609-615.

Celnik P (2015) Understanding and modulating motor learning with cerebellar stimulation. *Cerebellum (London, England)* 14:171-174.

Chen H, Hua SE, Smith MA, Lenz FA, Shadmehr R (2006) Effects of human cerebellar thalamus disruption on adaptive control of reaching. *Cerebral cortex (New York, NY : 1991)* 16:1462-1473.

Classen J, Liepert J, Wise SP, Hallett M, Cohen LG (1998) Rapid plasticity of human cortical movement representation induced by practice. *Journal of neurophysiology* 79:1117-1123.

Coxon JP, Peat NM, Byblow WD (2014) Primary motor cortex disinhibition during motor skill learning. *Journal of neurophysiology* 112:156-164.

Criscimagna-Hemminger SE, Donchin O, Gazzaniga MS, Shadmehr R (2003) Learned dynamics of reaching movements generalize from dominant to nondominant arm. *Journal of neurophysiology* 89:168-176.

D'Angelo E (2005) Synaptic plasticity at the cerebellum input stage: mechanisms and functional implications. *Archives italiennes de biologie* 143:143-156.

D'Angelo E (2014) The organization of plasticity in the cerebellar cortex: from synapses to control. *Progress in brain research* 210:31-58.

Daskalakis ZJ, Paradiso GO, Christensen BK, Fitzgerald PB, Gunraj C, Chen R (2004) Exploring the connectivity between the cerebellum and motor cortex in humans. *The Journal of physiology* 557:689-700.

Dayan E, Cohen LG (2011) Neuroplasticity subserving motor skill learning. *Neuron* 72:443-454.

Diedrichsen J, Hashambhoy Y, Rane T, Shadmehr R (2005) Neural correlates of reach errors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25:9919-9931.

Diedrichsen J, White O, Newman D, Lally N (2010) Use-dependent and error-based learning of motor behaviors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30:5159-5166.

Dimitrova A, de Greiff A, Schoch B, Gerwig M, Frings M, Gizewski ER, Timmann D (2006) Activation of cerebellar nuclei comparing finger, foot and tongue movements as revealed by fMRI. *Brain research bulletin* 71:233-241.

Donchin O, Francis JT, Shadmehr R (2003) Quantifying generalization from trial-by-trial behavior of adaptive systems that learn with basis functions: theory and experiments in human motor control. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23:9032-9045.

Donchin O, Rabe K, Diedrichsen J, Lally N, Schoch B, Gizewski ER, Timmann D (2012) Cerebellar regions involved in adaptation to force field and visuomotor perturbation. *Journal of neurophysiology* 107:134-147.

Doyon J, Benali H (2005) Reorganization and plasticity in the adult brain during learning of motor skills. *Current opinion in neurobiology* 15:161-167.

Dum RP, Strick PL (2003) An unfolded map of the cerebellar dentate nucleus and its projections to the cerebral cortex. *Journal of neurophysiology* 89:634-639.

Duque J, Hummel F, Celnik P, Murase N, Mazzocchio R, Cohen LG (2005) Transcallosal inhibition in chronic subcortical stroke. *NeuroImage* 28:940-946.

Dyke K, Kim S, Jackson GM, Jackson SR (2016) Intra-Subject Consistency and Reliability of Response Following 2 mA Transcranial Direct Current Stimulation. *Brain stimulation*.

Eccles JC, Llinás R, Sasaki K (1966) The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *The Journal of physiology* 182:268-296.

Evrard HC, Craig AD (2008) Retrograde analysis of the cerebellar projections to the posteroventral part of the ventral lateral thalamic nucleus in the macaque monkey. *The Journal of comparative neurology* 508:286-314.

Floyer-Lea A, Matthews PM (2005) Distinguishable brain activation networks for short- and long-term motor skill learning. *Journal of neurophysiology* 94:512-518.

Fox CA, Barnard JW (1957) A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibres. *Journal of Anatomy* 91:299-313.

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66:198-204.

Galea JM, Jayaram G, Ajagbe L, Celnik P (2009) Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29:9115-9122.

Galea JM, Vazquez A, Pasricha N, de Xivry JJ, Celnik P (2011) Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. *Cerebral cortex (New York, NY : 1991)* 21:1761-1770.

Galvez V, Alonzo A, Martin D, Loo CK (2013) Transcranial direct current stimulation treatment protocols: should stimulus intensity be constant or incremental over multiple sessions? The international journal of neuropsychopharmacology 16:13-21.

Gao Z, van Beugen BJ, De Zeeuw CI (2012) Distributed synergistic plasticity and cerebellar learning. Nature reviews Neuroscience 13:619-635.

Gilbert PF, Thach WT (1977) Purkinje cell activity during motor learning. Brain research 128:309-328.

Grasselli G, Hansel C (2014) Cerebellar long-term potentiation: cellular mechanisms and role in learning. International review of neurobiology 117:39-51.

Graydon FX, Friston KJ, Thomas CG, Brooks VB, Menon RS (2005) Learning-related fMRI activation associated with a rotational visuo-motor transformation. Brain research Cognitive brain research 22:373-383.

Grodd W, Hulsmann E, Lotze M, Wildgruber D, Erb M (2001) Sensorimotor mapping of the human cerebellum: fMRI evidence of somatotopic organization. Human brain mapping 13:55-73.

Hadipour-Niktarash A, Lee CK, Desmond JE, Shadmehr R (2007) Impairment of retention but not acquisition of a visuomotor skill through time-dependent disruption of primary motor cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:13413-13419.

Haith AM, Krakauer JW (2013) Model-based and model-free mechanisms of human motor learning. *Advances in experimental medicine and biology* 782:1-21.

Heiney SA, Kim J, Augustine GJ, Medina JF (2014) Precise control of movement kinematics by optogenetic inhibition of Purkinje cell activity. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34:2321-2330.

Heise KF, Niehoff M, Feldheim JF, Liuzzi G, Gerloff C, Hummel FC (2014) Differential behavioral and physiological effects of anodal transcranial direct current stimulation in healthy adults of younger and older age. *Frontiers in aging neuroscience* 6:146.

Herzfeld DJ, Pastor D, Haith AM, Rossetti Y, Shadmehr R, O'Shea J (2014) Contributions of the cerebellum and the motor cortex to acquisition and retention of motor memories. *NeuroImage* 98:147-158.

Hoover JE, Strick PL (1999) The organization of cerebellar and basal ganglia outputs to primary motor cortex as revealed by retrograde transneuronal transport of herpes simplex virus type 1. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 19:1446-1463.

Horvath JC, Vogrin SJ, Carter O, Cook MJ, Forte JD (2016) Effects of a common transcranial direct current stimulation (tDCS) protocol on motor evoked potentials found to be highly variable within individuals over 9 testing sessions. *Experimental brain research* 234:2629-2642.

Huang VS, Haith A, Mazzoni P, Krakauer JW (2011) Rethinking motor learning and savings in adaptation paradigms: model-free memory for successful actions combines with internal models. *Neuron* 70:787-801.

Hummel FC, Steven B, Hoppe J, Heise K, Thomalla G, Cohen LG, Gerloff C (2009) Deficient intracortical inhibition (SICI) during movement preparation after chronic stroke. *Neurology* 72:1766-1772.

Imamizu H, Kuroda T, Miyauchi S, Yoshioka T, Kawato M (2003) Modular organization of internal models of tools in the human cerebellum. *Proceedings of the National Academy of Sciences of the United States of America* 100:5461-5466.

Imamizu H, Miyauchi S, Tamada T, Sasaki Y, Takino R, Putz B, Yoshioka T, Kawato M (2000) Human cerebellar activity reflecting an acquired internal model of a new tool. *Nature* 403:192-195.

Ishikawa T, Tomatsu S, Tsunoda Y, Lee J, Hoffman DS, Kakei S (2014) Releasing dentate nucleus cells from Purkinje cell inhibition generates output from the cerebrocerebellum. *PloS one* 9:e108774.

Ito M (2001) Cerebellar long-term depression: characterization, signal transduction, and functional roles. *Physiological reviews* 81:1143-1195.

Itō M (1984) *The cerebellum and neural control*: Raven Pr.

Jacobs KM, Donoghue JP (1991) Reshaping the cortical motor map by unmasking latent intracortical connections. *Science (New York, NY)* 251:944-947.

Jayaram G, Galea JM, Bastian AJ, Celnik P (2011) Human locomotor adaptive learning is proportional to depression of cerebellar excitability. *Cerebral cortex* (New York, NY : 1991) 21:1901-1909.

Jayaram G, Tang B, Pallegadda R, Vasudevan EV, Celnik P, Bastian A (2012) Modulating locomotor adaptation with cerebellar stimulation. *Journal of neurophysiology* 107:2950-2957.

Joiner WM, Brayanov JB, Smith MA (2013) The training schedule affects the stability, not the magnitude, of the interlimb transfer of learned dynamics. *Journal of neurophysiology* 110:984-998.

Jorntell H (2016) Cerebellar physiology: links between microcircuitry properties and sensorimotor functions. *The Journal of physiology*.

Jorntell H, Ekerot CF (2003) Receptive field plasticity profoundly alters the cutaneous parallel fiber synaptic input to cerebellar interneurons in vivo. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23:9620-9631.

Jorntell H, Hansel C (2006) Synaptic memories upside down: bidirectional plasticity at cerebellar parallel fiber-Purkinje cell synapses. *Neuron* 52:227-238.

Kapreli E, Athanasopoulos S, Papathanasiou M, Van Hecke P, Keleki D, Peeters R, Strimpakos N, Sunaert S (2007) Lower limb sensorimotor network: issues of somatotopy and overlap. *Cortex; a journal devoted to the study of the nervous system and behavior* 43:219-232.

Karni A, Meyer G, Jezard P, Adams MM, Turner R, Ungerleider LG (1995) Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377:155-158.

Kashiwabuchi N, Ikeda K, Araki K, Hirano T, Shibuki K, Takayama C, Inoue Y, Kutsuwada T, Yagi T, Kang Y, et al. (1995) Impairment of motor coordination, Purkinje cell synapse formation, and cerebellar long-term depression in GluR delta 2 mutant mice. *Cell* 81:245-252.

Kim S, Ogawa K, Lv J, Schweighofer N, Imamizu H (2015) Neural Substrates Related to Motor Memory with Multiple Timescales in Sensorimotor Adaptation. *PLoS biology* 13:e1002312.

Kleim JA, Barbay S, Nudo RJ (1998) Functional reorganization of the rat motor cortex following motor skill learning. *Journal of neurophysiology* 80:3321-3325.

Kleim JA, Vij K, Ballard DH, Greenough WT (1997) Learning-dependent synaptic modifications in the cerebellar cortex of the adult rat persist for at least four weeks. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17:717-721.

Kleim JA, Hogg TM, VandenBerg PM, Cooper NR, Bruneau R, Remple M (2004) Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24:628-633.

Kleim JA, Barbay S, Cooper NR, Hogg TM, Reidel CN, Remple MS, Nudo RJ (2002) Motor learning-dependent synaptogenesis is localized to functionally reorganized motor cortex. *Neurobiology of learning and memory* 77:63-77.

Krakauer JW, Ghez C, Ghilardi MF (2005) Adaptation to visuomotor transformations: consolidation, interference, and forgetting. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25:473-478.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD (1993) Corticocortical inhibition in human motor cortex. *The Journal of physiology* 471:501-519.

Kuper M, Thurling M, Stefanescu R, Maderwald S, Roths J, Elles HG, Ladd ME, Diedrichsen J, Timmann D (2012) Evidence for a motor somatotopy in the cerebellar dentate nucleus--an FMRI study in humans. *Human brain mapping* 33:2741-2749.

Lehericy S, Benali H, Van de Moortele PF, Pelegrini-Issac M, Waechter T, Ugurbil K, Doyon J (2005) Distinct basal ganglia territories are engaged in early and advanced motor sequence learning. *Proceedings of the National Academy of Sciences of the United States of America* 102:12566-12571.

Lepage JF, Morin-Moncet O, Beaulieu V, de Beaumont L, Champoux F, Theoret H (2012) Occlusion of LTP-like plasticity in human primary motor cortex by action observation. *PloS one* 7:e38754.

Liebetanz D, Nitsche MA, Tergau F, Paulus W (2002) Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain : a journal of neurology* 125:2238-2247.

Lopez-Alonso V, Cheeran B, Rio-Rodriguez D, Fernandez-Del-Olmo M (2014) Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain stimulation* 7:372-380.

Lopez-Alonso V, Fernandez-Del-Olmo M, Costantini A, Gonzalez-Henriquez JJ, Cheeran B (2015) Intra-individual variability in the response to anodal transcranial direct current stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* 126:2342-2347.

Lu X, Miyachi S, Ito Y, Nambu A, Takada M (2007) Topographic distribution of output neurons in cerebellar nuclei and cortex to somatotopic map of primary motor cortex. *The European journal of neuroscience* 25:2374-2382.

Luaute J, Schwartz S, Rossetti Y, Spiridon M, Rode G, Boisson D, Vuilleumier P (2009) Dynamic changes in brain activity during prism adaptation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29:169-178.

Malinow R, Mainen ZF, Hayashi Y (2000) LTP mechanisms: from silence to four-lane traffic. *Current opinion in neurobiology* 10:352-357.

Mapelli L, Pagani M, Garrido JA, D'Angelo E (2015) Integrated plasticity at inhibitory and excitatory synapses in the cerebellar circuit. *Frontiers in cellular neuroscience* 9:169.

Marr D (1969) A theory of cerebellar cortex. *The Journal of physiology* 202:437-470.

Martin TA, Keating JG, Goodkin HP, Bastian AJ, Thach WT (1996) Throwing while looking through prisms. I. Focal olivocerebellar lesions impair adaptation. *Brain : a journal of neurology* 119 (Pt 4):1183-1198.

Medina JF, Mauk MD (2000) Computer simulation of cerebellar information processing. *Nature neuroscience* 3 Suppl:1205-1211.

Medina JF, Lisberger SG (2008) Links from complex spikes to local plasticity and motor learning in the cerebellum of awake-behaving monkeys. *Nature neuroscience* 11:1185-1192.

Middleton FA, Strick PL (2001) Cerebellar projections to the prefrontal cortex of the primate. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 21:700-712.

Miller AD, Brooks VB (1982) Parallel pathways for movement initiation of monkeys. *Experimental brain research* 45:328-332.

Morton SM, Bastian AJ (2004) Prism adaptation during walking generalizes to reaching and requires the cerebellum. *Journal of neurophysiology* 92:2497-2509.

Morton SM, Bastian AJ (2006) Cerebellar contributions to locomotor adaptations during splitbelt treadmill walking. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26:9107-9116.

Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M (2001) Role of the human motor cortex in rapid motor learning. *Experimental brain research* 136:431-438.

Murase N, Duque J, Mazzocchio R, Cohen LG (2004) Influence of interhemispheric interactions on motor function in chronic stroke. *Annals of neurology* 55:400-409.

Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, Tergau F (2003) Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *Journal of cognitive neuroscience* 15:619-626.

Nitschke MF, Kleinschmidt A, Wessel K, Frahm J (1996) Somatotopic motor representation in the human anterior cerebellum. A high-resolution functional MRI study. *Brain : a journal of neurology* 119 (Pt 3):1023-1029.

Nomura T, Takeshima T, Nakashima K (2001) Reduced pre-movement facilitation of motor evoked potentials in spinocerebellar degeneration. *Journal of the neurological sciences* 187:41-47.

Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM (1996) Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16:785-807.

Orban de Xivry JJ, Marko MK, Pekny SE, Pastor D, Izawa J, Celnik P, Shadmehr R (2011) Stimulation of the human motor cortex alters generalization patterns of motor learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31:7102-7110.

Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M (1995) Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *Journal of neurophysiology* 74:1037-1045.

Penhune VB, Doyon J (2005) Cerebellum and M1 interaction during early learning of timed motor sequences. *NeuroImage* 26:801-812.

Penhune VB, Steele CJ (2012) Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. *Behavioural brain research* 226:579-591.

Perez MA, Lungholt BK, Nyborg K, Nielsen JB (2004) Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Experimental brain research* 159:197-205.

Pinto AD, Chen R (2001) Suppression of the motor cortex by magnetic stimulation of the cerebellum. *Experimental brain research* 140:505-510.

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarah E, Celnik PA, Krakauer JW (2009) Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proceedings of the National Academy of Sciences of the United States of America* 106:1590-1595.

Richardson AG, Overduin SA, Valero-Cabre A, Padoa-Schioppa C, Pascual-Leone A, Bizzi E, Press DZ (2006) Disruption of primary motor cortex before learning impairs memory of movement dynamics. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26:12466-12470.

Rijntjes M, Buechel C, Kiebel S, Weiller C (1999) Multiple somatotopic representations in the human cerebellum. *Neuroreport* 10:3653-3658.

Rioult-Pedotti MS, Friedman D, Donoghue JP (2000) Learning-induced LTP in neocortex. *Science* (New York, NY) 290:533-536.

Rioult-Pedotti MS, Donoghue JP, Dunaevsky A (2007) Plasticity of the synaptic modification range. *Journal of neurophysiology* 98:3688-3695.

Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP (1998) Strengthening of horizontal cortical connections following skill learning. *Nature neuroscience* 1:230-234.

Rispal-Padel L, Cicirata F, Pons C (1982) Cerebellar nuclear topography of simple and synergistic movements in the alert baboon (*Papio papio*). *Experimental brain research* 47:365-380.

Robertson EM, Pascual-Leone A, Miall RC (2004) Current concepts in procedural consolidation. *Nature reviews Neuroscience* 5:576-582.

Rosenkranz K, Kacar A, Rothwell JC (2007) Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 27:12058-12066.

Rossini PM et al. (2015) Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* 126:1071-1107.

Sainburg RL, Wang J (2002) Interlimb transfer of visuomotor rotations: independence of direction and final position information. *Experimental brain research* 145:437-447.

Savin DN, Morton SM (2008) Asymmetric generalization between the arm and leg following prism-induced visuomotor adaptation. *Experimental brain research* 186:175-182.

Schlerf JE, Verstynen TD, Ivry RB, Spencer RM (2010) Evidence of a novel somatopic map in the human neocerebellum during complex actions. *Journal of neurophysiology* 103:3330-3336.

Schlerf JE, Galea JM, Bastian AJ, Celnik PA (2012) Dynamic modulation of cerebellar excitability for abrupt, but not gradual, visuomotor adaptation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 32:11610-11617.

Schlerf JE, Galea JM, Spampinato D, Celnik PA (2015) Laterality Differences in Cerebellar-Motor Cortex Connectivity. *Cerebral cortex (New York, NY : 1991)* 25:1827-1834.

Schonewille M, Gao Z, Boele H-J, Veloz MFV, Amerika WE, Simek AAM, De Jeu MT, Steinberg JP, Takamiya K, Hoebeek FE, Linden DJ, Hugarir RL, De Zeeuw CI (2011) Reevaluating the Role of LTD in Cerebellar Motor Learning. *Neuron* 70:43-50.

Seidler RD (2006) Differential effects of age on sequence learning and sensorimotor adaptation. *Brain research bulletin* 70:337-346.

Seidler RD, Purushotham A, Kim SG, Ugurbil K, Willingham D, Ashe J (2005) Neural correlates of encoding and expression in implicit sequence learning. *Experimental brain research* 165:114-124.

Shadmehr R, Krakauer JW (2008) A computational neuroanatomy for motor control. *Experimental brain research* 185:359-381.

Shadmehr R, Smith MA, Krakauer JW (2010) Error correction, sensory prediction, and adaptation in motor control. *Annual review of neuroscience* 33:89-108.

Shinoda Y, Futami T, Kano M (1985) Synaptic organization of the cerebello-thalamo-cerebral pathway in the cat. II. Input-output organization of single thalamocortical neurons in the ventrolateral thalamus. *Neuroscience research* 2:157-180.

Smith MA, Shadmehr R (2005) Intact ability to learn internal models of arm dynamics in Huntington's disease but not cerebellar degeneration. *Journal of neurophysiology* 93:2809-2821.

Steele CJ, Penhune VB (2010) Specific increases within global decreases: a functional magnetic resonance imaging investigation of five days of motor sequence learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30:8332-8341.

Stefan K, Wycislo M, Gentner R, Schramm A, Naumann M, Reiners K, Classen J (2006) Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. *Cerebral cortex* (New York, NY : 1991) 16:376-385.

Stoodley CJ, Schmahmann JD (2009) Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. *NeuroImage* 44:489-501.

Torriero S, Oliveri M, Koch G, Lo Gerfo E, Salerno S, Ferlazzo F, Caltagirone C, Petrosini L (2011) Changes in cerebello-motor connectivity during procedural learning by actual execution and observation. *Journal of cognitive neuroscience* 23:338-348.

Tseng YW, Diedrichsen J, Krakauer JW, Shadmehr R, Bastian AJ (2007) Sensory prediction errors drive cerebellum-dependent adaptation of reaching. *Journal of neurophysiology* 98:54-62.

Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I (1995) Magnetic stimulation over the cerebellum in humans. *Annals of neurology* 37:703-713.

van Kan PL, Horn KM, Gibson AR (1994) The importance of hand use to discharge of interpositus neurones of the monkey. *The Journal of physiology* 480 (Pt 1):171-190.

Wang J, Sainburg RL (2004) Interlimb transfer of novel inertial dynamics is asymmetrical. *Journal of neurophysiology* 92:349-360.

Wang J, Sainburg RL (2005) Adaptation to visuomotor rotations remaps movement vectors, not final positions. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25:4024-4030.

Weiner MJ, Hallett M, Funkenstein HH (1983) Adaptation to lateral displacement of vision in patients with lesions of the central nervous system. *Neurology* 33:766-772.

Werhahn KJ, Taylor J, Ridding M, Meyer BU, Rothwell JC (1996) Effect of transcranial magnetic stimulation over the cerebellum on the excitability of human motor cortex. *Electroencephalography and clinical neurophysiology* 101:58-66.

Wessel MJ, Zimmerman M, Timmermann JE, Heise KF, Gerloff C, Hummel FC (2016) Enhancing Consolidation of a New Temporal Motor Skill by Cerebellar Noninvasive Stimulation. *Cerebral cortex* (New York, NY : 1991) 26:1660-1667.

Wolpert DM, Kawato M (1998) Multiple paired forward and inverse models for motor control. *Neural networks : the official journal of the International Neural Network Society* 11:1317-1329.

Wu T, Kansaku K, Hallett M (2004) How self-initiated memorized movements become automatic: a functional MRI study. *Journal of neurophysiology* 91:1690-1698.

Wymbs NF, Bastian AJ, Celnik PA (2016) Motor Skills Are Strengthened through Reconsolidation. *Current biology : CB* 26:338-343.

Yang Y, Lisberger SG (2014) Role of plasticity at different sites across the time course of cerebellar motor learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34:7077-7090.

Ziemann U, Rothwell JC, Ridding MC (1996) Interaction between intracortical inhibition and facilitation in human motor cortex. *The Journal of physiology* 496 (Pt 3):873-881.

Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D (2004) Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24:1666-1672.

Vitae

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